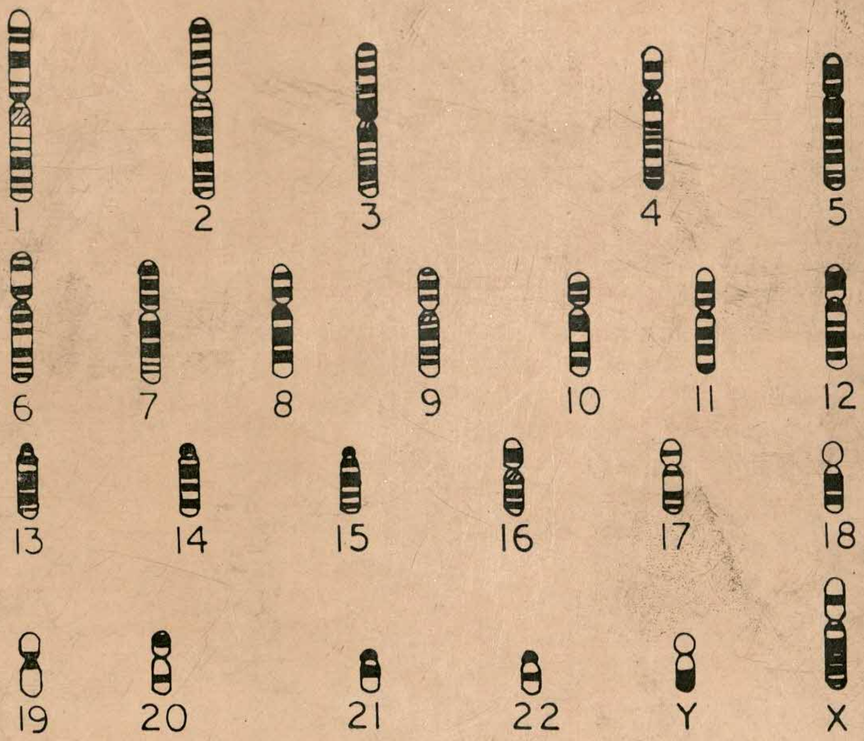

SECOND EDITION

# Chromosomes

Archana Sharma

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# CHROMOSOMES

SECOND EDITION

**ARCHANA SHARMA**

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# PREFACE TO THE SECOND EDITION

After the publication of the first edition in 1976, every year brought new developments in methods for the study of chromosomes and their components. Application of the improved techniques led to a more in-depth understanding of the structure and behaviour of chromosomes. Though the basic ideas regarding the chromosomal alterations and karyotype evolution remained, yet these were modified in the light of subsequent obervations. The role of polymorphisms, genetical and chromosomal, as related to the environmental factors, both internal and external, became clearer.

The gene concept has undergone radical changes with the identification of the split genes and the different types of DNA sequences—repeated and unique. The chromosome has emerged as a complex system, with inbuilt mechanisms to control its different components in accordance with the behavioural pattern required. The control is dynamic, to suit different situations imposed through external causes. This dynamicity is expressed in changes in the structure and behaviour of the DNA, for example, in response to the requirements of reproduction, differentiation and evolution.

In this edition, the chromosomal basis of differentiation and senescence has been included, as also the different aspects of mutagenesis and carcinogenesis. Most of the chapters have been revised thoroughly, with additional information. Several have been rewritten and several more added.

As in the previous edition, the students of my postgraduate classes and research workers have formed the initial audience for these lectures. I have drawn heavily on the material required for them in formulating the topics. I have freely used the ideas of my husband, Professor A.K. Sharma, often without his knowledge and the opinions of my colleagues. To all of them I am really grateful. My debt is heaviest to my immediate family members for their forbearance to my neglect.

Calcutta, 1985 ARCHANA SHARMA

# PREFACE TO THE FIRST EDITION

This book has grown out of a course of lectures given to the postgraduate students in genetics, at the University of Calcutta, intended for both general and special classes. I was concerned with explaining the development of the science of chromosomes, its various ramifications and future prospects

These articles were of course written individually to meet different immediate ends. They however follow a basic unity of purpose, inasmuch as they deal with the chromosome—its structure and behaviour, both normal and aberrant.

With the recent rapid changes in curricula, a student is exposed to the laws of heredity and the concept of DNA as the material basis of heredity at the school-leaving stage. Undergraduate courses in cytogenetics, in most universities of this country, cover the morphology of the chromosome, its behaviour during division, alterations both at genic and genomic levels, namely mutation and polyploidy, their induction and application. Many excellent treatises are available on genetics or cytogenetics all over the world. This book is not meant to add to their ranks.

Based on the infrastructure gained by the student at the undergraduate level, my attempts are to present the chromosomes as living, dynamic entities against the backdrop of evolution and speciation, to the comparatively advanced worker. For this purpose, the book has been divided into five parts. Since the advancements in chromosome science have been, of necessity, a triumph of advancements in methodology and microscopy, the principles underlying these topics and their future evolution form the first section. The second section deals with the nature of chromosome, in all its varied components. The third part is concerned with the gene and its behaviour. Alterations in the chromosome structure and their fate compose the fourth section.

The concept of the karyotype and its impact on speciation and the evolution of living forms are the subjects for the fifth section. The epilogue summarises, in brief, the present concept of the chromosome and prospects of some future developments.

The objective of this book is to present *chromosome science*, in its fundamentals and advancements, and the implications of its study in various facets of differentiation and evolution. Abnormality in chromosome behaviour has led on the one hand to the evolution of new forms, and on the other to congenital diseases, including malignancy. Examples have been drawn from all eukaryotic systems, animal, plant and human.

The topics will be of use to the postgraduate students, research workers and teachers interested in chromosomes and their many-faceted behaviour, in both plant and animal kingdoms. Greater emphasis has been laid on the directions in which chromosome science is proceeding with the potential of future achievements, rather than on the relatively mundane topics with which the students are already familiar.

I would like to acknowledge my indebtedness to the University Grants Commission, New Delhi for kindly granting me a book-writing project and to the National Book Trust, India for kindly subsidising the final product. My grateful thanks are due to the reviewers of the book, Dr B.P. Pal, FRS, President, Indian National Science Academy, New Delhi; Dr M.S. Swaminathan, FRS, Director General, Indian Council of Agricultural Research, New Delhi and Professor G.K. Manna, Head of the Department of Zoology, University of Kalyani. These eminent scientists, in spite of their very busy schedules were kind enough to assess the manuscript and help me with their very valuable comments. My gratitude should also be expressed to my past successive batches of students for being the unwitting guinea pigs in the execution of this work and to my family and my close associates, for their forbearance. It would perhaps be superfluous to thank my husband, Professor A.K. Sharma, Head, Department of Botany, University of Calcutta, as well, for the constant use I have made of his thoughts and works in this book, often without his knowledge.

Calcutta, 1976 ARCHANA SHARMA

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# SECTION I
# CHROMOSOME IDENTIFICATION

CHAPTER I: 1

# INTRODUCTION

IN the evolution of any branch of science, development of techniques has been a major factor. Chromosome study is no exception. Indeed, the phenomenal achievements in the study of the structure and function of chromosome have been principally due to two-fold advancements: in the field of instrumentation and in the field of methodology.

The birth of chromosome science came at a later stage of growth of the science of genetics. From his hybridisation experiments on different forms of pea, Mendel had formulated certain laws regarding the basic ratios followed in the transmission of characters from parents to the offspring. The regularity with which these characters were passed on. he attributed to certain factors located in the germ cells of each organism which are perpetuated · from generation to generation. Several other discoveries were made about at the same time—which, all unknown to him—later laid the foundations for his principles of heredity. From salmon sperm, Miescher—a biochemist—had extracted a substance—the *nuclein,* which he regarded as a universal component of the sperm. An embryologist—Hertwig—had. in the sea urchin, followed the union of the sperm nucleus with the egg nucleus during fertilisation to form a new nucleus. It remained for Flemming, the geneticist, to observe certain rod-like bodies, termed the *chromosome* or *colour body* within the nucleus. These chromosomes—he observed—are present in all cells. They divide equationally in the somatic cells and reductionally in the germ cells. The term *gene* was employed by Johansen, a plant breeder, for certain hypothetical physicochemical units within the cells, which form the material basis of heredity. The turn of the present century saw the amalgamation of these scattered observations into a comprehensive whole. The bits of the jigsaw puzzle formed an understandable picture when the genes of Johansen were found to be indeed factors following the Mendelian ratios. They are located on the chromosomes, which undergo reductional division during the formation of gametes, regain their somatic number through fusion of two nuclei during fertilisation and are composed of nuclein or more precisely, nucleoprotein.

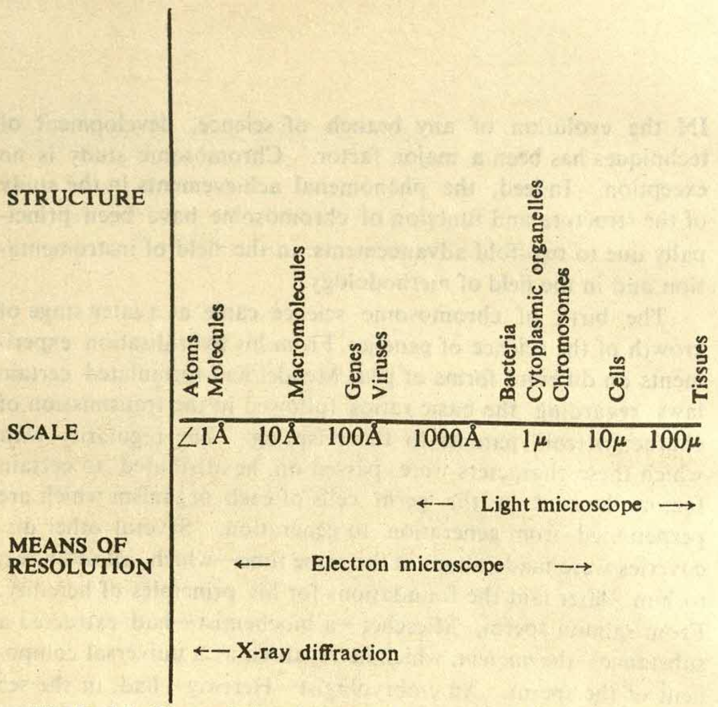

**Fig. I : 1.1 The resolution of biological components (after Lewis KR and John B 1963 *Chromosome marker* Churchill, London)**

PROKARYOTE CHROMOSOME: In the study of chromosome structure, a sharp demarcation exists between the chromosomes of prokaryotes like bacteria and viruses and those of the eukaryotes (see Table I: 1.1). The chromosome of the former is essentially a genophore, composed principally of a long chain DNA molecule. It has been studied in detail with the aid of electron microscopy and X-ray diffraction patterns as well as through recombination and mutation experiments. The manifestation of differentiation is not far removed from the initial stage of gene action in such a chromosome. The very simplicity of its organisation prevents its observation through light microscopy.

**TABLE I : 1.1**

**Characteristics of DNA in prokaryotic and eukaryotic cell types**

| *Prokaryotic* | *Eukaryotic* |
|---|---|
| Primarily haploid | Primarily diploid |
| DNA uncomplexed | DNA complexed with proteins forming chromosomes |
| DNA nonlocalised in the cell cytoplasm | DNA localised primarily within the nucleus of the cell |
| No morphologic stages in DNA replication | DNA replication described by mitotic cycle consisting of specific cytologic stages |
| DNA often found as a closed circle | DNA found in linear chromosome |
| Replication not associated with cellular organelles | Replication and separation of chromosome associated with cellular organelles called centrioles |

CHROMOSOME OF THE EUKARYOTES is a much more complex structure with at least four components—the two nucleic acids ribose and deoxyribose, and basic and non-basic proteins. The dynamic nature of the chromosome—with the changes involved in the different phases of its development and behaviour—adds

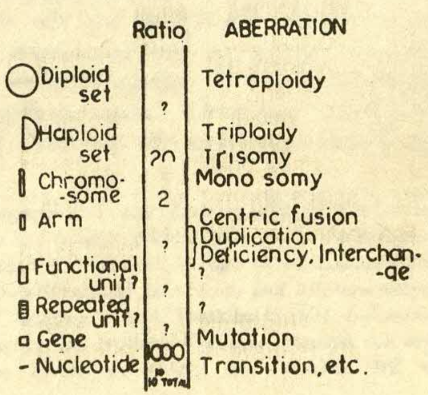

Fig. I : 1.2 Hierarchy of chromosomes.

to its complexity. The reaction initiated at the gene level is separated by a series of steps from its final phenotypic expression. Though presumably the genic composition of the chromosome complement remains constant in all cells of an organism, yet the variations in chromosome behaviour in relation to differentiation in different organs of the individual indicate that there should be a corresponding variability in the composition of the chromosome structure. The methodology has evolved accordingly. The principal factor in the study of chromosomes *in situ* is to accentuate the difference between them and the surrounding media. This can be achieved by differential alterations in the respective optical densities, through chemical treatment and differential staining. The different properties of the chromosomes, the other nuclear and cellular bodies and cytoplasm, are utilised in devising means to identify them individually. In this process, both cytochemical and biophysical techniques have been employed.

CHAPTER I: 2

# IMPROVEMENTS IN INSTRUMENTATION*

## Improvements in microscopy

Improvement in microscopy has been the first aspect in the development of instrumentation for chromosome science. Progressive specialisation from the earliest simple microscope to the compound one has permitted progressively a clearer observation of chromosomal details. The adoption of sophisticated optics, from simple lenses to aplanatic high power and oil immersion, from achromatic to apochromatic and planapochromatic objectives, has resulted in greater resolving power. The present-day compound light microscope for research is able to distinguish details of chromosomes much below $1\mu$. Phase contrast lenses have aided in observing the structure in living cells. Phase and interference microscopes change phase differences between cellular components into intensity differences by modifying the phases of the light that pass through different parts of the visual field. Both rely on interference between two beams of light. The phase microscope uses the specimen itself to separate the two beams while the interference microscope employs one optical system to split the beam. These types of microscopy have been utilised in observing the differences in cell structure through

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*(See Caspersson T and Zech L 1972 ed *Chromosome identification* Nobel symp *23* : Academic Press, New York; De Robertis EDP, Nowinski WW and Saez FA 1975 *Cell Biology* 7th ed Saunders Philadelphia; Gray P 1973 ed *Encyclopedia of Microscopy and Microtechnique* Van Nostrand, New York; Sharma A and Talukder G 1974 *Laboratory Procedures in Human Genetics* The Nucleus, Calcutta; Sharma AK and Sharma A 1980 *Chromosome Techniques—theory and practice* 3rd ed Butterworths London).

converting the phase differences between cellular components into intensity differences. A more recent tool, the fluorescence microscope, utilises the property of autofluorescence of intracellular components to distinguish them or alternatively, identifies them by secondary fluorescence due to the adherence of a labelled or fluorescent dye to a particular component. When observed through an ordinary microscope, the fluorescence emitted by a fluorescent specimen is rather weak so that the light source has to provide powerful excitation in the blue region. A phase contrast image can be superimposed on the fluorescent one for a combined study.

A FLUORESCENCE MICROSCOPE requires two special sets of filters, (i) the *exciter* filters absorbing the light of longer wavelength emitted by the source which would otherwise cover up the fainter fluorescence of the object under observation and (ii) the *barrier* filters absorbing the short wave component of the light emitted by the excited object, which appears self-luminous due to the long-wave fluorescence radiation emitted by it. The most adequate light source for ultraviolet rays is a carbon arc fitted with an electromagnetic field or a superpressure mercury or Xenon lamp. The only limitation of this method is the gradual fading of fluorescence on exposure to ultraviolet rays. This method has been used effectively for identification and quantitative estimation of cell constituents. Its spectacular utility in chromosome study was demonstrated in 1969 by Caspersson and Zech. Chromosomes, on being stained with quinacrine dihydrochloride (atebrin) or a derivative, quinacrine mustard, were found to fluoresce in alternate light and dark bands. These banding patterns served to distinguish each chromosome pair of the human karyotype and were constant and specific for a particular organism. Spectrophotometric measurements of the intensities show a correlation with the relative DNA localisation and content (see later under "banding patterns").

MICROSCOPE ACCESSORIES have attained progressive complexity to meet the demands of clearer resolution of cellular details. The earliest form of illumination was daylight focussed through a mirror. Earlier artificial illuminators were ordinary desk lamps with inside frosted bulbs, a conical flask filled with copper sulphate solution providing the filter. For research work, the present-day illuminator fulfils a two-fold objective: the field of view should be evenly illuminated and the back lens of the objective should be filled with the light as completely as possible when the condenser iris is open and the condenser is in its uppermost position. Most research microscopes have attachable substage illuminators or built-in pinpoint illuminators which act by the Köhler method, that is, by starting precise control of the light rays at the source. For observation as well as photography, different sets of filters are used, inserted between the lamp and the condenser. The two principal types of filter available are Wratten type and Interference band filters. The main principle of their use is that filters of the same or similar colour as the specimen reduce the contrast while those of complementary colours increase the contrast.

The earliest method of recording chromosome was through camera lucida drawings at table magnification. Drawing appliances of greater simplicity and better resolution were devised with improvement in microscopy. As drawings of karyotypes of chromosomes were gradually replaced by photographic records, various models of photomicroscopes were produced by different optical manufacturers. A present-day photomicroscope is fully equipped for automatic photography of the cells being observed in the microscopic field. Arrangements for projection of metaphase plates were first made on a ground glass screen for exhibiting to a number of viewers simultaneously. Later development resulted in a magnified projection of the desired plate on a television screen, where the intensity of the image can be varied as required and each chromosome can be identified individually.

PHASE CONTRAST MICROSCOPY is now used for routine study of various aspects of the structure and movement of chromosomes in the living cell. Microscope interferometry has permitted the measurement of the changes of the amount of protein and DNA in the nuclei as well as in the chromosomes from cell cultures. It has also been used to measure the total protein of isolated nuclei. Cell division has been filmed by using the interference system with excellent colour effects. Phase contrast microscopy is adequate for routine observations while interference microscopy is used principally for measuring cell constituents.

**POLARISATION MICROSCOPY** depends on the birefringence against polarised light shown by certain objects of complex molecular arrangement. It gives an indication of the physical heterogeneity of the structure at the molecular level. The microscope—in addition to the usual accessories of a light microscope—is fitted with polarising elements having a low intensity carbon or a high-pressure mercury arc as the illuminator. It has been particularly helpful in studying the structure of the mitotic spindle and the changes it undergoes during cell division.

**X-RAY MICROSCOPY** allows the resolution of molecular patterns of biological materials below 10Å, where electron microscopy is relatively ineffective. Different properties of x-rays, such as diffraction, emission and absorption are utilised in studying the chemical and physical nature of chromosomes. Diffraction pattern is analysed by passing a beam of x-rays through the object. The rays are scattered through diffraction by atoms. The emerging rays diverge according to the pattern of diffraction and are recorded on a photographic plate. The symmetry and pattern of the object are determined on the basis of the angle and intensity of the diffraction patterns. This method has aided in the establishment of the double helix configuration of the DNA molecule and the mode of arrangement of its different components. It is a very useful tool for the quantitative and the qualitative assessment of the structure and behaviour of chromosomes since the molecular interaction between chromosomes and x-radiation follows certain fixed principles.

**ELECTRON MICROSCOPY** has facilitated the study of chromosome structure at the submicroscopic level. Electron microscopes are designed with electrical or magnetic fields adequately shaped to refract electrons, producing the desired image, much in the same manner as light rays are reflected or refracted by lenses and mirrors in the light microscope. The electron image is then transformed to a visible light image on the fluorescent screen. The magnification thus attained is very high. Its essential constituents, assembled vertically, are: an illuminating system, a specimen chamber, an objective lens, intermediate and projecting lenses, and a viewing chamber with facilities for photographing electron images, maintained in a vacuum. The source of electrons forming the illuminating system is supplied by a hot tungsten cathode filament. The electrons are focussed on the material by an electromagnetic condenser lens. Similar objective lenses collect the electrons from the material and form a magnified image. It is then projected by electromagnetic eyepiece and projecting lenses onto a fluorescent screen. To allow the passage of electrons through a material and for its good resolution, ultrathin sectioning is required. Various types of special ultramicrotomes have been devised for this purpose.

The accessories, like special glass knife for section-cutting, jeweller's saws for trimming, mounting grids with a backing film, have been developed in response to the demand for obtaining suitable ultrathin sections required for electron microscopy.

Electron microscope studies have been invaluable in understanding the ultrastructure of the chromosome in different phases of its behaviour. Almost all aspects of chromosome organisation have been analysed, from the nature of the fibrils in the half-chromatids to the mechanism of gene action and the role of the nuclear membrane in nucleo-cytoplasmic transfer.

Electron microscopy is being increasingly applied for obtaining information on chromosome relationship and behaviour. An application has been in the construction of pachytene idiograms based on length and other characteristics of synaptonemal complexes.

## Autoradiography

Autoradiography, at the light microscope level, is based on the principle that if a photographic emulsion is brought into contact with radioactive material, the ionising radiation will so convert the emulsion as to show spots at certain points after being developed. Radioactive substances are introduced into the tissue either in a given chemical form or tagged with certain metabolic precursors. The radioactive molecule, at the site of its location, following treatment, can be identified by several methods, including digestion by enzymes, selective staining, extraction of different constituents and precipitation. In the stripping film method, the radioactivity treated chromosome preparation—usually a squash, smear or section on a slide—is covered with a sensitive emulsion having a bottom layer of gelatin against a glass plate. In an alternative schedule, emulsion is applied in the liquid form as a thin layer on the tissue. The slide is kept in dark for a certain period to permit the radiation emitted to activate the silver halide crystals in the emulsion. A latent image is thus formed which can be developed to denote the location and intensity of the radioactive material. When the radioactive substance is tagged with a metabolic precursor, the distribution of grains in the autoradiograph indicates the distribution of the precursors, outlining the metabolic path.

Autoradiography has been used extensively in the interpretation of controversial aspects of chromosome structure and metabolism. For example, tritium-labelled thymidine, with low energy β-particles, is used in analysing the chromosome thread and in proving the semi-conservative nature of DNA replication at chromosome level. The special properties of the heterochromatic segment, including the late replication of its DNA thread, have been shown by autoradiography, as also the fact that DNA synthesis takes place in interphase. The incorporation of labelled nucleosides like FUDR and BUDR in the chromosomes has demonstrated that DNA forms the fundamental skeleton of the chromosome and that any change in it may lead to gene mutation or chromosomal alteration. Its most significant contribution has been in elucidating the dynamicity of the chromosome structure during differentiation in different phases of development and growth.

### High resolution autoradiography

Autoradiography, combined with electron microscopy, has aided in the correlation of ultrastructure with function. An insight in the macromolecular pattern and specific behaviour of biological units varying between 10 and 20Å has been obtained. The method involves the use of tritiated compounds of high specificity and more refined schedules for processing.

### Microspectrophotometry under ultraviolet light

Microspectrophotometry under ultraviolet light aids in the quantitative estimation of cellular nucleoprotein due to the characteristic absorption of purine and pyrimidine from the nucleic acid at 2650Å. UV light can clarify unstained living cells, due to its strong absorption by nucleoprotein, nucleic acids showing absorption at 260 nm and proteins at about 280 nm. To measure changes in the quantity of nucleic acid and protein, the microscope with UV light is generally used as a spectrophotometer. The monochromatic beam of UV light is spilt into two beams one falling directly on a blank photoelectric cell and the other passing through the UV microscope to another photoelectric cell. The material is placed in the path of the beam passing through the microscope. The resultant reduction in its intensity is measured by counting the difference in the photoelectric currents yielded by the two beams. Several models of ultramicrospectrophotometers are now available with variations and innovations, including automatic manipulator for slides, automatic recording devices and computer analysis. This method is being utilised to study cell changes *in vivo*, to determine the base constituents of nucleic acids, extracted from isolated chromosome segments and for quantitation of different cell and chromosome components.

### Microspectrophotometry under visible light

Microspectrophotometry under visible light uses the absorption curves of coloured substances to help in the chemical identification, localisation and quantitation of chromosomal ingredients. The measurements are made of materials stained with different chromosomal dyes like feulgen, methyl green-pyronin and others which specifically stain individual components. The microscope is fitted with suitable accessories for measuring the amount of absorbed and transmitted light, including a photometer unit with photomultiplier tubes, rotating photometer mount, a projecting light source and a monochromator. Difference in the deflection of a recording galvanometer between the material and a blank space gives the amount of transmitted light, from which the extinction value can be calculated. The density of the tissue observed is estimated from readings taken with two wavelengths. Further improvements include automatic recording and interpretation of the data.

This procedure is useful in the quantitative correlation of DNA with the number of genomes present and also the distribution of DNA at different phases of development and growth. Since it is based principally on the intensity of the stain, it will have greater application with the discovery of new staining methods for specific cell constituents.

Further modifications of the ultramicrospectrophotometer involve a rapid scanning type with arrangement for preselection of measuring field. Other models are also being produced.

### Automated chromosome analysis

Automation in chromosome analysis is a recent development in this field. It shows certain advantages over the manual techniques, namely, a much higher speed, resulting in lower cost per cell analysed and quantitative measurements not otherwise possible. There are three aspects of the problem—of selecting suitable metaphase spreads and of the analysis of karyotypes from such spreads through counting and accurate measurement. The latter aspects have gained more importance. The criteria adopted in such methods include the optical density, chromosome size, relative measurements of the arms and also other morphological characters. The chromosomes are divided into different categories on the basis of the criteria chosen and scanned for these characters.

In one of the earlier methods, photographic negatives are scanned in a scanning instrument automatically and recorded in a programmed computer. At first the scanner detects the plate, searches out the individual chromosomes, analyses each chromosome as a spectrum of components, which are recorded. Alternatively, optical density measurements of a metaphase spread are recorded automatically in a digital form on magnetic tape and stored in computer memory. The data, when required, is produced in a picture of symbols chosen by their apparent greyness. In such cases, a suitable metaphase is selected by the worker and the instrument scans and records it automatically.

### Automatic interactive systems

Automated methods are also being developed for the detection of mitotic chromosome spreads, which compare favourably in speed with manual methods. With the evolution of the banding pattern techniques, *complex interactive systems* are needed to produce adequate results. The systems evolved may be applied to slide preparations on the microscope or to photographic prints or negatives. A typical setup consists of a computer, high resolution flying spot scanner, a mechanical scanner with a very great memory system, a microscope scanning system and an interactive communication system. It recognises banding patterns and can output a photographic image of an automatically constructed karyotype or draw density curves for each chromosome in the form of a karyotype. In a combined manual-automatic system, a metaphase spread is focussed under a microscope by an operator. The optical image is converted by the scanner into a numerical form. The image is sampled at regularly spaced points. At each point optical density or fluorescent intensity is quantitatively measured and given a value corresponding to a level of greyness. The image of the cell is thus converted into digits through a scanner directed from the microscope. The analysis of the chromosomes thus obtained can be presented to the human operator according to the form required by him, as a high resolution compos'te karyotype, a set of profile wave forms or a table with measurement data like length, area, arm ratio, density, with a picture of the original metaphase spread.

The primary goal of computer-oriented chromosome analysis is to produce an interactive automated system, giving the same efficiency as the human cytogeneticist using conventional microscopic and photographic techniques but with significantly lower cost and less time. It may be employed in routine work involving large population screening for genetic aberrations. The combination of an automated system for finding metaphases with that for identifying chromosomes from banding patterns has given some very promising results. However, the preparation of chromosome spreads for analysis and the interpretation of the data processed by the computer, are still under the complete control of the cytogeneticist, and likely to remain so, for several years to come.

The development of techniques for *banding pattern analysis of chromosomes* led to the corresponding innovation of *suitable instruments* or the modification of existing ones for their measurement. The four principal fields, where special biophysical methods are required are :

(i) objective recording of banding patterns with very high optical resolution;
(ii) rapid instrumentation for pattern recording;
(iii) identification of chromosome regions; and
(iv) rapid identification of aberrations.

*Instruments for banding pattern measurements*: Though devised for analysis of fluorescence patterns, yet these instruments can be applied, with equal precision and with suitable alterations, to banding patterns produced by the other techniques. A high resolution ultramicrofluorometer can measure fluorescence distribution curves directly on chromosomes, after scanning the plate under phase contrast. The optical resolution of this method is very close to the physical limits. The data is presented as graphs on an XY-recorder or punched onto a magnetic tape. which can be later "read". A combined densitometer-reflectometer has been devised to record fluorescence intensities of chromosome segments from a photographic print instead of the original metaphase plate. This method is much more rapid. The data thus obtained can also be digitised and punched onto a magnetic tape which can be directly evaluated at a computerised analysis. For faster identification work, a television camera is used to scan the photograph. The image is processed electronically so that the picture of the chromosome can be observed on a screen. Detailed comparison can be made between the patterns given by different banding techniques through two TV-cameras with zoom lenses, focussing two different images of two preparations on a single moniter screen. Further

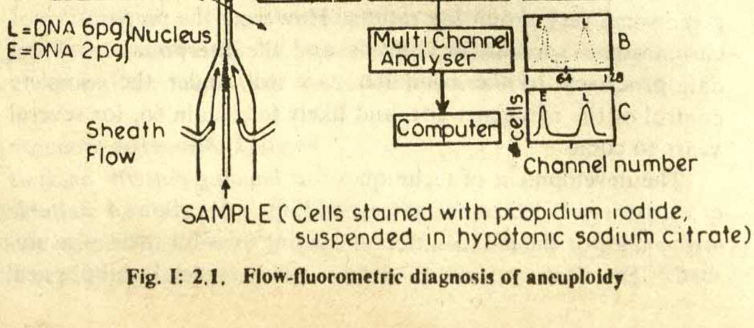

Fig. I: 2.1. Flow-fluorometric diagnosis of aneuploidy

sophistication has led to automatic recording of other parameters as well, as for example flow-fluorometric analysis of aneuploidy (Fig. I: 2.1).

## Applications of Chromosome Study are of Use:

In breeding practices
In raising new forms
In monitering for environmental mutagens
In identifying syndromes due to chromosomal anomalies
In identifying carriers of such diseases
In cancer studies

*Microscopy used include:*

Ordinary light microscopy
Phase and interference microscopy
Polarisation microscopy
X-ray microscopy
Fluorescence microscopy
Light microscope autoradiography
Electron microscopy
High resolution autoradiography
Microspectrophotometry

*Automated integrated analysis.*

CHAPTER I:3

# IMPROVEMENTS IN METHODOLOGY

FOR the study of the structure of chromosomes under the light microscope refinements of methodology have been a major factor. The principal difference between plant and animal cell is the presence of a cellulose cell wall in the meristematic cells of the former. This difference necessitates certain modifications in techniques otherwise similar in other aspects. The compactness of the plant tissue, as compared to animals, presents serious difficulties in the scattering of chromosomes within a restricted space.

## A. Chromosome morphology

DIVISIONAL STAGES: The most convenient stage for studying chromosome morphology is during the divisional cycles, both mitotic and meiotic. The metaphase during mitosis gives the easiest interpretation of chromosome number and structure, since the chromosomes are highly condensed and clearly observable as distinct entities. With the present-day squash techniques, they appear well-scattered against a background of solidified cytoplasm and the constriction regions are well-marked. The nature of the chromosome thread is, however, better observed during anaphase separation, through dissolving the outer matrix by a short exposure to concentrated nitric acid vapour. The meiotic stages serve on the other hand to illustrate the different aspects of chromosome behaviour, including pairing, crossing-over and recombination. The pachytene chromosome is an elongated body with well-marked chromomeres. The members of a pair can be matched easily during this phase. Such comparison between pachytene chromosomes often uncovers small deletions and inversions which may be otherwise overlooked in mitotic chromosomes. The lampbrush chromosomes are found as bivalents in the oocytes of amphibians with large yolk-filled eggs. They are up to 800$\mu$ long at diplotene and resemble a brush with long symmetrical loops emerging from the longitudinal axis. The size of these chromosomes and their peculiar nature make them a useful material for the study of chromatids and their subunits. The large polytene chromosomes of the dipteran salivary gland, particularly of *Drosophila* have been used extensively in the study of chromosome structure and behaviour, from gene mapping, observations of structural aberrations to the mechanics of RNA transcription and gene dosage.

### SOURCE OF MATERIAL FOR CHROMOSOME STUDY

The first prerequisite for chromosome study is to obtain a large number of cells in the dividing stage, particularly at metaphase. Any tissue of any eukaryotic organism at an actively dividing state may yield the different divisional configurations. In the higher plants, the root-tips, the shoot-tips and the leaf-tips are the sources for the study of the somatic chromosomes. Amongst the algae, the entire young vegetative filament can be studied. It is an excellent material for the observation of naturally synchronised division. Meiotic stages may be studied from the reproductive cells, during the reduction division. These range from the pollen or spore mother cells of the higher plants to the ascus amongst the fungi. Reduction division during megasporogenesis is more difficult to analyse due to the relative inaccessibility of the tissue concerned. The pollen grain is an excellent material for haploid mitotic complement analysis during the division into vegetative and reproductive nuclei. In certain cases, this division can be followed in the pollen tube after artificially germinating it in sucrose or other media. The endosperm tissue gives a triploid (3n) number and is a convenient material for study. Amongst the pteridophytes, the leaf-tips, sori and entire gametophyte tissue of the prothallus can be processed for chromosome study.

The choice is more limited in the animals, principally due to make-up of the animal body. Amongst the lower groups, the very young larvae and larval tails are suitable for observation.

The salivary glands of the insects are widely used. The meiotic studies are made from dividing gonadal cells either used directly or after mincing, depending on the texture and thickness. The germinal epithelium, spleen and lymph nodes provide good sources for direct study of cell division. Amongst the vertebrates, the bone marrow and later the blood are the most widely used materials. The male gamete formation is easily observed as the tissue is obtained by puncturing or biopsy from the gonads. Particularly in the higher mammals, the female germ cell division reaches the dictyotene in the embryonic condition and the later stages of the division take place only when an ovum enters its maturation cycle. The difficulty in obtaining such cells without a major operation results in their inavailability for routine work. Embryonic tissue is used, whenever available. In man particularly, the study of abortuses has yielded a wealth of useful data. Amniotic fluid and chorionic tissue, obtained through amniocentesis, are being progressively utilised to detect the genetical anomalies—chromosomal and biochemical—in the unborn foetus.

TISSUE CULTURE: Certain tissues, which normally show comparatively little or no division, may be stimulated to divide rapidly when cultured *in vitro*. In higher mammals, such tissues include blood, skin and fascia lata. The cultures may be short term, as for bone marrow or small tissue biopsies, or longer, as for peripheral blood. The most time-consuming method is that of studying chromosomes from cultures of fibroblasts and other tissues. Different types of culture media—both natural and synthetic—are available to give the tissue optimum environmental conditions for division and growth. The degree of divisional activity of mononuclear cells in peripheral blood—the most common and easily available source in cold and warm blooded animals—is considerably enhanced in the presence of a mitogenic agent—phytohaemagglutinin—extracted from red kidney bean, in the culture. The small lymphocytes are converted into blast cells when cultured in presence of PHA or a similar mitogenic agent in antilymphocytic serum, antigens and homologous leucocytes leading to high mitotic activity in the latter. The maximum response usually occurs by the third day, when the culture is harvested. PHA is the principal mitogenic agent in use. It has the dual properties of selectively agglutinating and sedimenting mature erythrocytes and of inducing division in leucocytes. Other mitogenic agents include tuberculin, tetanus toxoid, certain plant extracts like concanavalin A, but none of them is an effective substitute for PHA as yet.

In fibroblast cultures, the tissues used are generally skin, fascia lata, gonadal and most foetal tissues. They are set up as primary cultures as pieces or suspensions in a synthetic medium supplemented by serum and in most cases a growth stimulant, like embryo extract. After adequate growth, subcultures are started. When processing for chromosome preparations, partial synchrony in the divisions is obtained by changing the medium or the subculture.

Chromosome studies from organ culture in plants are relatively less in vogue since, as mentioned earlier, mitosis can be studied directly from different rapidly growing plant parts. In plant tissue culture, the only natural medium available is coconut milk where free nuclear division can be observed. For successful cultures, normally synthetic media, supplemented with vitamins and hormones, are employed. Despite the fact that a plant cell, even in the most evolved groups, shows totipotency, that is, the capacity of evolving during differentiation into any type of tissue, yet a plant tissue, on culture, first forms a callus —a mass of undifferentiated cells—from which the different organs later arise. Chromosomes from callus tissue are relatively difficult to analyse, though they can be studied through suspension cultures and give variable numbers.

However, methods have been evolved to study chromosomes from differentiated plant tissues. The endopolyploid nature of permanently differentiated tissue was first observed by Huskins, who suggested that in such nuclei, the chromosome thread replicates but the two strands do not separate. The ensuing condition resembles the polytene chromosome of the *Drosophila* salivary glands. Several chemicals, usually a component or a precursor of nucleic acids, are found to be capable of inducing division in these permanent nuclei. Feeding with nucleic acid salts also induces division though the frequency is not high, due to the low penetrability of the large molecules into the tissue. Different hormones, including indolyl butyric acid as well as growth promoters like gibberellic acid, kinetin and 2, 4,-didedosophenoxy-

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acetate are capable of inducing division.

Of the nucleic acid components, best results were obtained with sugars indicating a deficiency in metabolic DNA to be responsible for the normal state of endoreplication. On supplying the component required, the chromosomes undergo separation. The degree of endoreduplication is progressively higher in the more mature tissues, as shown by the large size of the prophase nuclei and the number of chromosomes when they are brought to metaphase. Possibly, in the non-dividing differentiated nuclei, a DNA thread, after acting as a template for the formation of a fixed number of messenger RNAs, requires renewal. It undergoes replication during interphase, but to maintain the harmony of the differentiated body the reduplicated chromosome or segment is unable to proceed to anaphase for separation. As a result, the number of threads continues to multiply, leading to a polytenic constitution.

SEPARATION OF CELLS: The next stage in chromosome preparations is to secure a clear cytoplasmic background along with cell separation. The latter step is essential for plants due to the compactness of the tissue and the presence of a middle lamella. In animals, the use of enzymes like trypsin or collagenase and EDTA is adequate to obtain suitable cell suspensions even from fibroblast tissues. In plants, heating or warming in dilute acids serves the dual purpose of dissolving the middle lamella and clearing the cytoplasm. However, acid treatment often results in diminished stainability and in the recent methods of chromosome banding, interferes in the formation of specific bands. In such cases, specific enzymes like pectinase, clarase and snail stomach cytase are preferable. In order to remove cell inclusions like starch, oil and cytoplasmic granules, the tissue can be effectively treated in dilute solutions of enzymes like diastase and lipase. Chlorophyll in leaf-tip preparations is removed by prolonged overnight fixation in a fixative containing ethyl alcohol or acidulated alcohol.

PRETREATMENT: Pretreatment in a suitable chemical is required to obtain separation of individual chromosomes, clarification of chromosome segments and a large frequency of metaphase stages. The principle underlying the process is viscosity change in the cytoplasm caused by the chemical concerned. Spindle formation is known to depend on a viscosity balance between the spindle constituents and the cytoplasm of a cell. Change in the cytoplasmic viscosity destroys the spindle, so that the chromosomes are released from any binding force within the cell. On squashing or smearing, the pressure causes the chromosomes to scatter. At the same time, the segments of the chromosome, due to the viscosity change, undergo differential hydration. Stretching of these segments, during squashing, against a solidified plasma, causes the constriction regions to be well clarified. Another result of spindle inhibition is metaphase arrest, through which a large number of dividing nuclei come to metaphase and are unable to proceed further in the absence of spindle fibres. A number of chemicals are known to be effective as pretreatment agents, the most well-known one being colchicine—an alkaloid extracted from *Colchicum autumnale*. Other commonly used pretreatment chemicals are acenaphthene, chloral hydrate, coumarin and its derivatives, aesculine, isopsoralene, oxyquinolene, α-bromonaphthalene and *p*-dichlorobenzene. In addition, most radiomimetic chemicals can be successfully employed for obtaining well-scattered metaphase plates if applied at suitably low concentrations. At such dosages spindle formation is inhibited, plasma is nearly solidified and the freed chromosomes are scattered on squashing against a semi-solid background. The effects of different chemicals on plant chromosomes may be classified into lethal, narcotic and subnarcotic. The clarification of chromosome morphology is included within the subnarcotic effects. Even pond or tap water, and to some extent water distilled in a metallic retort have been observed to disturb spindle activity. The degree of viscosity change required to bring about spindle upset is a specific character. Therefore the concentration and period of treatment, as also the chemical used, are specific for the organism concerned. Colchicine, *p*-dichlorobenzene and oxyquinolene have a comparatively wider field of application than the others. Cold temperature assists in the viscosity change and is required for most of these chemicals, except coumarin. The use of pretreatment chemicals has simplified the study of chromosome structure through eliminating the earlier time-consuming microtomy schedules, since squashing or air-drying is an essential step for the former. The pretreating effect is associated with specific chemical changes. For example, psoralene, which differs from isopsoralene in the presence of a furan ring, has no effect, as also isocolchicine—an isomer of colchicine. Colchicine is applied in the form of colcemid to obtain metaphase arrest in mammals either in the culture or injected into the animal. The latter procedure is being gradually discarded due to its injurious effects.

HYPOTONIC TREATMENT: Hypotonic treatment for chromosome spread is based on the same principle as pretreatment, or swelling the cells with consequent dispersion of chromosomes. It is used extensively in leucocyte culture, particularly in human and mammalian materials. An effective hypotonic solution must be of low osmotic pressure and low ionic strength. The solutions used range from water, hypotonic sodium citrate and sodium chloride to dilute balanced salt solutions and culture medium. The period and temperature of application vary with the material used.

FIXATION: The next step after pretreatment and/or hypotonic treatment is fixation. Fixatives are used to preserve the forms of cells and their contents as closely as possible to their living state and to render them resistant to further changes caused by subsequent processing. The properties desired in an ideal fixative are those of precipitating chromatin, of immediate killing, of checking the autolysis of proteins and of enhancing the basophilia of chromosomes. A very wide range of fixatives, both metallic and non-metallic, and their combinations for chromosome preparation are available. However, advancements in the process of fixation have principally involved simplification of the earlier schedules. Addition of metals and other agents serves to increase the basophilia of chromosomes but may also affect depolymerisation of the nucleic acids. Therefore the complex fixing mixtures evolved by earlier workers are being progressively discarded, in light microscopic and cytochemical studies, in favour of chilled alcohol and neutral formalin and in case of mammalian cells, acetic acid-methyl alcohol mixture. Metallic fixatives aid staining by adsorption.

STAINING: Staining of chromosomes is carried out to make them visible under the light microscope. Different cell constituents react differently to a single dye. The factors influencing the degree of colouration depend on the chemical affinity between the constituent and the dye, the density of the constituent and its permeability by the dye. With certain stains, like methylene blue, cell division can be observed in tissue culture without killing the tissue. However, such *vital* stains have a very limited application. Most chromosome stains are *non-vital* and impart colour to the chromosomes in the fixed tissue by certain chemical agents which are insoluble in the chromosome substance. The principal dyes used to stain chromosomes are synthetic organic dyes, mainly derived from coal tar. The colour of a dye is due to a chemical configuration known as the *chromophore* while the colour retained by the tissue is due to certain chemical configurations in the dye molecule itself, known as the *auxochrome.* Chromosomal stains are termed *acidic* or *basic* on the basis of their chemical nature and behaviour. In some cases, as in orcein, they are amphoteric. For differential staining, it is the practice to overstain the chromosome, followed by removal of the excess stain, also known as differentiation. Staining can also be performed progressively by gradual increase in the intensity of the colour. It is claimed to be mainly brought about through adsorption and may be accelerated by mordanting with salts of di- and trivalent metals which form compounds, attaching the dye to the tissue. Many chromosome stains are available with varying degrees of specificity for DNA.

Of all the different staining methods employed for the study of chromosomes, the Feulgen reaction is regarded as the most specific one. It is based on the principle that on controlled hydrolysis with normal HCl, the aldehyde group of the sugar moiety of DNA is liberated through separation of the purine-containing fraction. The unmasked aldehyde groups undergo Schiff's reaction with fuchsin sulphurous acid to give a typical magenta colouration of the chromosomes. The results however are variable, requiring strict control. Other triphenyl methane dyes have been worked out, which give results comparable to the feulgen test. A widely used chromosome stain is orcein. Staining in this amphoteric dye involves its condensation at the polypeptide linkage of the chromosomal protein. Other single stains for chromosomes are carmine and lacmoid, used dissolved in acetic or propionic acids. Crystal violet was widely used for staining chromosomes in paraffin section preparations, as also haematoxylin, which was outdated with the advent of squash preparations. A stain used extensively in mammalian chromosomes is Giemsa—a mixture of several dyes, including methylene blue and its oxidation products—azures A and B and eosin Y. The quality of the stain varies with regard to the proportion of the dye used. Polychrome methylene blue and Tetrachrome stain have components somewhat similar to Giemsa. Less well known are brazilin, carbol fuchsin, cresylecht violet, Leishman stain, safranin, thionin and toluidine blue, with limited application. Double staining methods were evolved for specific staining of chromosome components as against other cell constituents. Feulgen staining, specific for DNA, which stains chromosomes magenta, was combined with light green to obtain a contrasting green cytoplasm and nucleolus. Pyronin-methyl green schedule was evolved for localisation of RNA and DNA in the tissue. It was originally based on the concept of the physical state of the two nucleic acids, the more highly polymerised DNA combining with methyl green. Though methyl green was claimed to combine with DNA due to its heavy cationic weight, yet the specificity of the method—in which the chromosomes stain green and the cytoplasm and the nucleolus pink—remains undisputed. Orange G-aniline blue combination, used for staining heterochromatin at the resting stage and telophase, is comparatively rarely used.

FLUOROCHROME DYES: Later additions to the family of stains are the fluorescent dyes. The design of instruments for high resolution ultrafluorometry permitted determination of DNA down to a theoretical level of $10^{-16}$ g of DNA or approximately 200 genes. Caspersson conceived the idea that various chromosomal segments may be selectively stained with an appropriate fluorescent agent that would bind selectively to bases in chromosomal DNA. The first fluorochrome studied was quinacrine mustard (QM) which gave intense fluorescence in the heterochromatic regions of chromosomes. Such bands formed the landmarks in a detailed analysis of the human karyotype. Other alkylating fluorochromes, including amino-acridine nitrogen mustards and a bisbenzimidazole derivative also produce similar bands in plant chromosomes. Non-alkylating fluorochromes like quinacrine, proflavine, and acriflavine give similar fluorescent banding in plant chromosomes. Acridine orange (AO) produces variable banding in plants while ethidium bromide gives reverse bands. The dye is usually applied to the chromosome preparation, either squashed or air dried and then mounted in a suitable medium and observed in a fluorescence microscope. The sites of dye attachment appear as fluorescent bands of differing intensities, against a dark background. All these dyes show a pronounced specificity for nucleic acids either because they react with DNA by alkylation of certain purine bases (QM) or because they bear positive charges (AO, proflavine, acriflavin) which allow them to interact with the DNA-phosphate backbone. It is further stabilized by the intercalation of the flat acridine rings between nucleic acid base pairs. Acridine orange (AO) is being increasingly used to study the strandedness of nucleic acids, particularly that of DNA in chromatin and chromosomes. After staining, the AO molecules are dispersed within the helix of a double-stranded DNA combining as a monomer without intermolecular interactions and fluoresce green, absorbing light at 500 nm. On the other hand stacks of AO molecules are formed combining as polymers on single-stranded DNA or RNA which fluoresces red absorbing light at 463 nm. A bisbenzimidazol derivative Hoechst 33258 shows a higher affinity for double-stranded DNA and can be used in the study of various chromosome segments with differential rates of reassociation following denaturation (see also Hecht F *et al.* 1974 In : *The Cell Nucleus* **2** : 34).

Base-specific anti-nucleoside antibodies, which react with specific bases of single-stranded DNA, like ±5-methyl cytosine have been utilised to obtain specific fluorescent banding on chromosomes. The fluorescent antiadenosine antibodies give a pattern resembling *Q*-bands while anticytidine produces the opposite type of bands, corresponding with *R*-bands. Certain chemicals, which form specific fluorescent conjugates with amino acids and proteins, like dansyl-chloride, indicate the distribution of proteins along a chromosome under the fluorescence microscope after suitable treatment.

A further development of the fluorochrome dyes is in the detection of sister chromatid exchanges. If cells are grown for two successive replication cycles in the presence of the base analogue BrdUrd and then stained with Hoechst 33258 or AO, the sister chromatids fluoresce differentially, making it possible to study the regions of exchange.

MOUNTING: To preserve the stained chromosome preparations, different mounting media are used. For air-dried slides, in most cases mounting is unnecessary since unmounted slides are more easily destained or restained as required. Temporary preparations can be mounted by adding a drop of the stain itself or its solvent to the squashed tissue, applying a coverglass over it and ringing the coverglass with paraffin or lacquer or a similar cementing material to prevent evaporation. When a preparation has to be maintained for long periods, all preservation media may be used for mounting, though the resinous ones are the most satisfactory. A desirable medium has a refractive index close to that of glass, dries quickly and checks the loss of stain in the preparation. Both natural and synthetic resins are in use. They are usually soluble in xylol. Therefore the preparation is dehydrated through immersion in alcohol grades followed by a period of treatment in xylol prior to mounting.

PROCESSING: An important step in chromosome methodology has been the transition from the earlier block preparation to the later squash or smear preparations. The previous method included fixation and embedding in a suitable medium like paraffin or colloidion to prepare blocks. They were subsequently sectioned, the embedding material removed and then stained. The process—in addition to being cumbersome and long-drawn out—suffered from the limitations of an inadequate number of metaphase plates, insufficiently scattered chromosomes and unresolved details. The development of the squash and smear techniques—where the tissue is pretreated and fixed, followed by smearing or squashing on a slide as the case may be—was a tremendous step forward in obtaining chromosome spreads of the desired clarity. Air-drying is followed for leucocyte cultures instead of smearing. Staining may precede or follow the process. The later evolution of the banding pattern analysis methods is based on subjecting the air-dried or squash preparations to different chemical treatments.

BANDING TECHNIQUES: The development of banding patterns followed the discovery in the 1970's that when chromosomes are stained with the fluorochrome quinacrine or its derivatives and viewed under ultraviolet light, they fluoresce or glow in alternate light and dark bands. Like fingerprints these fluorescent banding patterns are unique for each chromosome. Unlike fingerprints, however, the bands are generally consistent for a taxon except for minor variations. A number of other chemicals can also produce bands either identical with or different from the fluorescent ones, based on different principles. Such differential banding patterns, usually observed at specific regions on particular chromosomes, are being increasingly used for the identification of chromosomes. These bands are made visible through low and high intensity regions under the fluorescence microscope or as differentially stained areas under the light microscope. The principle of banding patterns through fluorochromes has been discussed earlier under "staining".

Methods to demonstrate Giemsa-stained banding patterns of mammalian chromosomes were described from different laboratories using basic principles of *in vitro* hybridisation of DNA. The earlier methods depended on alkaline denaturation followed by renaturation in an adequate buffer. Pardue MC and Gall JG (1970 *Science* **168**: 1356), in preparations for *in situ* hybridisation with mouse sat-DNA, noted that after autoradiography centromeric regions of mouse chromosomes showed more dense grains than chromosome arms and were considered to be heterochromatic. The same procedure was used to show the presence of such regions in human chromosomes with modifications. The areas of centric heterochromatin stained more intensely than the rest. It was suggested that these regions probably represented repetitive DNA. The presumed basis for differential staining under this technique was that renatured DNA appeared better able to combine with stain than partially denatured DNA. After denaturation by the different agents used, it was apparently the repetitive DNA which renatured most rapidly. The process of renaturation was halted optimally at a time when repetitive DNA had renatured but unique DNA had not. This specific staining was at first attributed to constitutive heterochromatin. However, later constitutive heterochromatin, though generally associated with satellite DNA in the mouse (that is, repetitive DNA, *not* the DNA of satellites), was seen to be actually a different entity and did not necessarily consist of short repetitive units.

Later schedules, referred to as methods for studying the *G*-banding are simpler, based on assumed differential denaturation only. The agents for producing bands are alkali used in different temperatures, several proteolytic enzymes and protein denaturing substances, including detergents, usually "anionic" ones. The process of differential processing used in these simplified *G*-band techniques seems to depend on the temperature, pH, ionic strength, divalent cation content and proteolytic enzyme content of the medium. A pattern of variable staining is observed after these treatments and after staining with Giemsa, which can be utilised effectively to identify individual chromosome pairs.

The chromosome band nomenclature, first adopted at the Paris Conference in 1971, recognised the following types of banding in human chromosomes:

*Q-band*: by quinacrine staining and fluorescence.

*G-band*: by staining with techniques using Giemsa and related stains after appropriate pretreatment.

*R-band or banding reverse to Q-bands*: by staining with Giemsa after heating to 87°C.

*C-bands*: for constitutive heterochromatin, demonstrated by the denaturation-reassociation technique.

*E-bands*: produced by enzymatic digestion, as classified by Lejeune (1973) show swollen and shrunken regions corresponding to the dark and faint regions of *G*-banding.

The same nomenclature can be applied to banding patterns observed in other eukaryotic chromosomes. Other banding patterns of chromosomes include:

*CT-bands*: the centromeric and telomeric segments show bands after treatment in barium hydroxide, incubation and staining in "Stains All" (4,5,4'5'-dibenzo-3,3'-diethyl-9-methylthiacarbocyanine bromide) in mammals.

*N-bands*: at nucleolus-organising regions, possibly due to acidic proteins in mammals.

*O-bands*: with orcein staining, mainly for plant chromosomes; both intercalary and centromeric heterochromatin show bands.

*Cd-bands*: where the centromeric dots were differentially stained;

*Hy-bands*: non-specific staining of heterochromatic segments.

It was initially suggested that centromeric heterochromatin appears in the *C*-bands and the intercalary heterochromatin in the *G* bands. The former is rich in highly repetitive satellite DNA while the latter is regarded to have non-repetitive poorly methylated DNA. Protein-DNA interactions are evidently important factors in all types of banding procedures. In *C*-banding the selective extraction of non-band DNA plays a major role but the intensity of Giemsa staining is enhanced by DNA-protein interactions. DNA in the *C*-bands is presumably resistant to extraction due to the associated proteins, without involving the idea of differential renaturation of repetitive DNA. In both *G* and *R* bands, base composition may be involved but DNA-protein interactions seem to play the major role. In *Q*-banding, negative or pale staining regions are due to the presence of nonhistone proteins binding in the small groove of DNA and preventing the intercalary binding of quinacrine. A similar mechanism may be responsible for *G*-banding (Comings DE 1974 *The Cell Nucleus* **1**: Academic New York). Neither *C*- nor *G*-banding appears to depend on the differential denaturation of DNA. Variations both in base ratios and overlying proteins are involved in the banding mechanisms. Denaturation or differential denaturation may influence to some extent *R*-banding. Even then, the method applied to demonstrate *R*-bands may affect protein binding sites in a different way.

Summarising the data available, the view that DNA is the important chromosome constituent in banding does not account for all the variants. Staining with Giemsa and other basic dyes depends to a considerable extent on the binding sites present in the DNA. The availability of these sites is controlled by the factor of their being blocked by protein. The role of proteolytic enzymes in producing banding indicates the importance of chromosomal protein in banding. *G*-bands, from detailed observation of chromosomal ultrastructure, are seen to produce a distortion of the normal distribution of chromosomal fibres and it is supported by the observation that divalent cations may play a role in banding. The band and interband regions are regarded to reflect the concentration and arrangement of nucleoprotein fibres. The idea that DNA-protein interactions are involved explains almost all the available evidences, since lysine-rich histones appear to bind preferentially to AT-rich DNA.

SISTER CHROMATID EXCHANGES represent the interchange of DNA strands between replication products at apparently homogenous loci. These exchanges are seen in cytological preparations of metaphase chromosomes and are used extensively to identify the carcinogenic and mutagenic effects on chromosomes. A number of known DNA damaging agents has been observed to cause significant increase in SCE frequency, typically at doses below those inducing increased chromosomal aberrations (Kitzmiller JB 1976 *Adv Genet* **18**: 315). These exchanges were first described by Taylor *et al* while demonstrating semiconservative replication of DNA threads through autoradiography. Sister chromatids were differentially labelled in cells which had undergone one cycle of $^3$H-dT incorporation, followed by a replication cycle in non-radioactive medium. This method was replaced by the BrdU-dye techniques. When BrdU is substituted in DNA, followed by staining by certain bound dyes, the fluorescence of the dye is quenched, as for example, of 33258 Hoechst, acridine orange and at alkaline pH 4,6-diamidinophenylindole (DAPI).

Light energy retained by these dyes induces the selective degradation of BrdU-substituted DNA, leading to a decrease in Giemsa staining. Further modifications are based on altering the schedule to utilise additional effects of BrdU on chromatin structure to obtain further differentiation. Comparison of SCE induction results with mutagenesis, carcinogenesis and unscheduled DNA synthesis data supports the extensive use of this method as an assay for mutagens and carcinogens. Very few cases of false negatives are on record. SCEs have been found to occur in certain human hereditary diseases, predisposing to neoplasia. Cells from patients with Bloom's syndrome (BS) show enhanced SCEs as well as exchange between different chromosomes, exhibited as quadriradial figures. This increased SCE frequency in BS cells can be reduced by fusing affected cells with cells from non-BS individuals. Xeroderma pigmentosum cells show abnormality in SCE frequency when exposed to uv light and indicate that SCEs are induced by unremoved DNA damage. Agents that can induce SCEs are able to induce chromosomal aberrations as well (see Latt SA, Schreck RR,

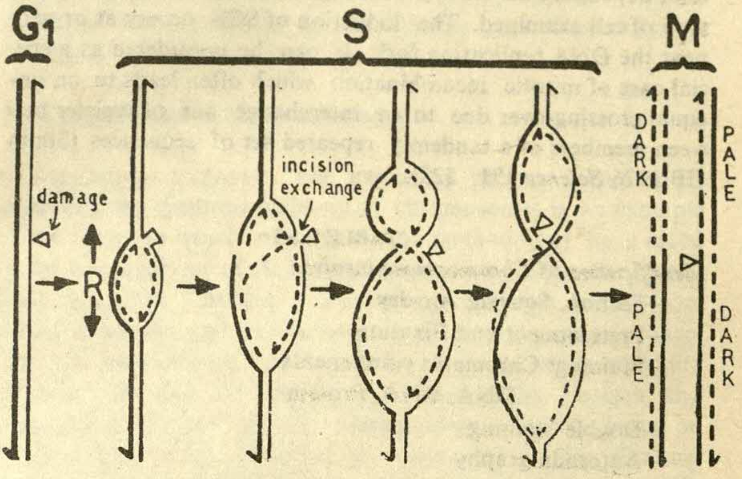

Fig. I: 3.1 Sister chromatid exchange (SCE) may result from a variety of different DNA lesions; develop during replication (S); and involve an exchange of duplexes and not half-strands.

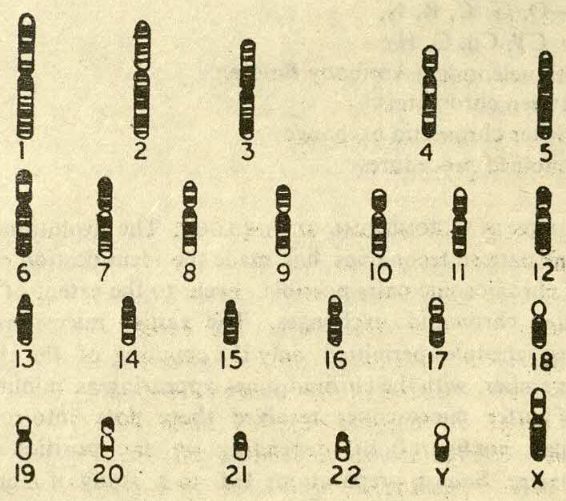

Fig. I: 3.2 Distribution of SCE along individual chromosomes in man.

Loveday KS and Shuler CF 1982 In: *Cytogenetic assays of environmental mutagens,* Allenheld, Osmun).

However, the relative frequency of the two may be quite different, depending on the type of DNA damage involved and the type of cell examined. The induction of SCE occurs at or very near the DNA replication fork. It can be considered as a special case of mitotic recombination which often leads to an unequal crossing-over due to an interchange out of register between members of a tandemly repeated set of sequences (Smith GP 1976 *Science* **191**: 528).

**TABLE I: 3.1**

*Identification of Chromosomes involves*

—Section, Squash, Air-dry
—Pretreatment and Fixation
—Staining: Chromatin components
DNA, RNA, Protein
—Double staining
—Autoradiography

*Identification of Segments involves*

Within chromosomes:
  Banding patterns
  —Q, G, C, R, N,
  CT, Cd, O, Hy
Antinucleoside—Antibody Binding
Between chromatids:
  Sister chromatid exchange
Combined procedures

EVOLUTION IN CHROMOSOME TECHNOLOGY: The evolution of the banding pattern techniques has made the identification of individual chromosome pairs possible, even to the extent of studying sister chromatid exchanges. The earlier microtomy and staining schedules permitted only the counting of the chromosome number, with the chromosomes appearing as minute dots. Use of better microscopes resolved these dots into rod, J or V-shaped configurations, depending on the position of the centromere. Squash preparations led to a study of morphological details and karyotype analysis. Banding pattern methods have resulted in the identification of individual chromosome segments with the possibility of the study of their behaviour and molecular configuration. The number of bands identifiable in the human karyotype is about 3000 at mid-prophase. A single structural band contains about 50 loops each 35 kb in length (Yunis JJ 1981 *Human Genet* **56**: 496).

### B. Chemical nature of chromosomes

CYTOCHEMICAL METHODS: The evolution of our present ideas regarding the chemical make-up of chromosomes is an example of the extent to which refinements of methodology have aided in the advancement of theoretical knowledge. After the identification of the "nuclein"—isolated by Miescher (1874) from nuclei of various animals—as the constituent of gene substance, the next breakthrough in the study of chromosome structure came in 1924 with the Feulgen test evolved by Feulgen and Rossenbeck for the specific identification of DNA, based on Schiff's reaction for aldehydes, as mentioned earlier. Gradual evolution of histochemical and cytochemical methods established the polynucleotide structure of the DNA molecule in association with the basic and non-basic proteins. The shift of technology from the cytogenetical level to the cytochemical one revealed the pattern of its organisation at the submicroscopical stage. In chemical terms, the chromosome may be visualised as a giant complex molecule made up of several less complex molecules, which in the eukaryotes, include at least two types of nucleic acids, DNA and RNA and two types of proteins. This study of chromosome structure has been aided through the biochemical methods of isolation of chromatin matter, cytochemical tests for localising the constituents *in situ*, the study of ultrastructure and the methods for quantitative analysis.

The cytochemical procedures include precise tests for nucleic acids and proteins in general and amino acids in particular at the sites of their location. They involve tests for specific components, as the Feulgen reaction for aldehydes and the Dische reaction for desoxy sugars. Base components of DNA, like purine and pyrimidine, can be best identified through ultraviolet absorption at 2650Å. The tests for phosphoric acids are not so precise. Methods for the simultaneous detection of DNA and

RNA are based on reactions either with sugar moiety or with the phosphoric groups, the most commonly used one in the latter set being the pyronin-methyl green technique. For the study of chromosomal proteins *in situ*, a number of chemical tests are available. They include the Sakaguchi reaction for arginine, tyrosine and tryptophane, based mainly on Millon's method and sulphydryl tests for proteins. These reactions for the localisation of individual chromosome components are usually preceded by specific digestion procedures to remove the other components which may interfere with the desired reaction. Enzymes can be located through different cytochemical methods at the loci of their occurrence. Alkaline phosphatase has been observed through the deposition of calcium phosphate or by the azodye technique involving precipitation of the alcoholic part of the phosphate ester. Modified procedures have been adopted for acid phosphatase. Cytochrome oxidase may be detected through the G Nadi reaction in which nuclei stain pink and the oxidase granules blue.

ISOLATION PROCEDURES: Chromosomes can be studied after isolation as well. They may be isolated either directly through micrurgical methods or indirectly by chemical treatment. The preparation may be fixed in 45% acetic acid and the chromosomes removed from the cell with the help of a needle. In unfixed preparations, the tissue or the cells are punctured and the chromosomes squeezed out in sugar medium. Alternatively, the chromosomes may be taken out with a needle after the cells are treated with enzyme, homogenised and centrifuged. In the chemical method, the chilled tissue is ground, extracted with saline, and the chromatin threads are isolated through differential and repeated centrifugation.

For the extraction of nucleic acids, different chemicals have been used, including acids like trichloracetic acid and perchloric acid. Enzyme digestion serves to locate a particular component in the cell. Those used for the digestion of nucleoproteins are nucleases –desoxyribose for DNA and ribose for RNA—or proteases—trypsin or chymotrypsin for basic proteins and pepsin for histone and more acidic proteins (see Sharma AK and Sharma A 1980 *Chromosome techniques* 3rd ed Butterworths London).

## C. Mapping of chromosomes

### SOMATIC CELL FUSION

(*i*) *In the mapping of chromosomes*, i.e., locating the positions of individual genes, somatic cell fusion has been an invaluable tool. Cells from two completely different cell lines, growing in culture, are fused together with the help of a virus, the *Sendai* virus, belonging to the para-influenza group. The virus is previously subjected to UV-irradiation, which destroys its ability to infect and grow in cells. The first cell hybrids, arising spontaneously, were discovered by Barski and his colleagues in 1960. *Sendai* virus was employed in 1962 by Okada to fuse mononucleate cells into giant cells with several nuclei. In 1965 Harris and Watkins developed the technique for fusing cells from widely different species into multinucleate hybrid cells, which have nuclei from both parents within a common cytoplasm. When the hybrid cell is a small one, containing only two nuclei, one from each parent, it may survive to complete a divisional cycle. The daughter cells, thus formed, have the complete chromosome complements of both parent nuclei within a single nucleus and can be maintained often in tissue culture for many years. Some of the more interesting crosses involving human cell lines are those with mouse, rat, chick, Chinese hamster and even mosquito. In the successive divisions of the hybrid cells, many of the human chromosomes are selectively lost but some remain. If a single human chromosome is finally left and the hybrid cell produces certain human proteins, it may be concluded that the genes for the synthesis of these proteins are located on that particular chromosome. This principle has been used in mapping the location of structural genes on human chromosomes. In practice, single hybrid cells are cultured to form independent clones, which continue to multiply, losing chromosomes in the process. The human chromosomes finally retained in each clone are identified through banding techniques. The clones are subjected to gel electrophoresis to distinguish the different human enzymes present. From a comparison of the human enzyme patterns and chromosome constitution of a large number of clones, the gene for synthesising a particular protein can be assigned to a particular chromosome. For example, the gene for thymidine kinase was assigned to a particular subgroup of the human karyotype by observing the elimination of chromosomes from a man × mouse hybrid culture. Thus individual chromosomes have been divided into bands to which groups of genes have been assigned. For such tests, three clinically important enzymes, G6PD, PKG and HGPRT, the lack of which is associated with different diseases, have been found to be controlled by three gene loci, located on a particular band of the X chromosome. Each of the more than 2000 bands at present seen on the 46 normal human chromosomes have several hundred or more of such gene loci.

Panels of hybrid cell clones have been assembled with various combinations of human chromosomes on constant mouse backgrounds or of mouse chromosomes on constant Chinese hamster backgrounds. In humans, both immunochemical techniques and electrophoretic fractionation of cell extracts on different gels have been applied to many constitutively expressed enzymes, structural proteins and cell surface markers. These mapped genes can also be tested for their possible linkage to traits which cannot be assayed in tissue culture. In this way, markers like the blood group antigens have been included in the genetic map.

This process of somatic cell hybridization, however, suffered from two major drawbacks (a) that it cannot be applied to the products of differentiated cells and (b) that it cannot give any information about the large part of mammalian genome that does *not* code for proteins. The next breakthrough in somatic gene mapping came by a combination of recombinant DNA methodology with somatic cell hybridisation.

(ii) *In combination with recombinant DNA technology*: DNA fragment mapping can be used to detect the sequence of a particular gene within the total number of about two million nucleotide base pairs of DNA present in the haploid genome of man as well as of mice and Chinese hamsters. In this *blotting* technique (Southern E 1975 *J Mol Biol* **98**: 503 and Wahl GM, Stern N and Stark GR 1979 *PNAS USA* **76**: 3663), chromosomal DNA is extracted from cells, purified to remove associated protein and digested with specific restriction endonucleases. These enzymes recognise short sequences of DNA, four to six nucleotides in length and cleave the DNA at the recognition sites. A large number of specific polynucleotide fragments are produced. These are separated by electrophoresis on agarose gel, and treated with alkali to separate out the nucleotide strands. The single strands are then blotted onto nitrocellulose paper. Recombinant DNA probes, produced artificially (Maniatis T, Fritsch EF and Sambrook J 1982 *Molecular Cloning* Cold Spring Harbor Lab) are radiolabelled, separated into single strands and incubated with the nitrocellulose paper. These reform a double helix, binding specifically to the DNA fragment and producing a radioactive band on the nitrocellulose.

Even if one nucleotide is altered in a recognition site, the restriction enzyme cannot cleave at that site and addition or removal of a few hundred bases would alter the electrophoretic mobility of a fragment. Using suitable restriction enzymes, it is thus possible to identify homologous genes from different species in a somatic cell hybrid. This method was first applied to hybrids of mouse and Chinese hamster somatic cells to locate the immunoglobulin K light chain on mouse chromosome 6 and has since then been used to map numerous human and mice genes on their respective chromosomes. Not all genes are easily cloned. But any gene or DNA sequence that can be cloned can also be localised on a chromosome. This method has been applied extensively in characterising multigene families and identifying dispersed gene families in mammals.

The application of recombinant DNA technology to somatic cell hybrids has been utilised to define a high density of genetic markers corresponding to a particular human chromosome or chromosomal region. For this purpose, genomic DNA fragments are isolated from a hybrid containing a fragment or a whole human donor chromosome in a rodent recipient cell and cloned into bacteriophage to generate a library. Of the recombinant phages, most will contain rodent DNA but a few will contain DNA fragments from the human chromosome. The latter are identified by transferring the DNA onto a nitrocellulose filter and hybridising with a radiolabelled probe corresponding to human reiterated sequences. This method has made it possible to isolate so far DNA fragments from human chromosomes 2, 3, 5, 6, 11, 12, 14, 16, 20 and X.

Thus recombinant DNA techniques have led to several rapid gene-mapping procedures, which have expedited the process of gene-cataloging. The products of differentiated cells, highly conserved genes and DNA sequences not expressed in normal issues are now easily mapped. The complementary mapping, using recombinant DNA probes derived from somatic cell hybrids to identify markers on particular chromosomes, has considerably advanced the genetic analysis of human and murine chromosomes (see also D'Eustachio P 1984 *American Scientist* **72**: 32).

(iii) *In cancer therapy*: When a normal and a malignant cells are fused together, the resulting hybrid cell is usually malignant. However, in certain cases, a normal cell can suppress the malignant properties of a malignant parent so that a non-malignant hybrid cell is formed. Hybrid cells containing the almost complete chromosome complements of both parents rarely give rise to tumours but those which have lost many chromosomes tend to be malignant. A possible explanation is that if malignancy is due to a chromosomal defect in the malignant cell strain, it is not expressed in the presence of a complete normal chromosome complement in the hybrid cell. When some of the normal chromosomes are eliminated, one or more of which may have been responsible for the suppression of malignancy, the hybrid cell exhibits malignant properties. The demonstration of the dominance of the normal cell over the malignant one can be used to advantage in experiments aimed at cancer therapy.

In cases of tumours produced by virus, a normal cell infected by tumour virus may permit the virus to replicate and destroy the host cell in the process. Such cell strains are referred to as *permissive.* Alternatively, the virus may cause transformation in the cell. In culture such cells undergo a process analogous to the formation of tumour cells from normal ones in the living animal. The virus cannot be detected after some cell generations though the property of the transformed cells does not change. Possibly in such cases a part of the viral genome is integrated in the host chromosome with which it replicates in synchrony, being carried on through successive cell divisions in the "masked" form. When such transformed cells are fused with permissive cells, the "masked" tumour virus is reactivated and begins to replicate. Cell hydridisation can thus also be employed in unmasking dormant tumour virus in tumour cells of suspected viral origin. The liberation of this virus from the integrated state in the host chromosome results in the loss of transforming capacity of the host cell and provides another possible aspect of cancer therapy (see chap II: 6).

(iv) *In the study of gene regulation:* Somatic cell hybridisation also has applications in the analysis of cell growth cycle and in the study of gene regulation and cell differentiation in animal cells. In heterokaryons formed by the fusion of actively growing cells, including tumour cell lines with highly differentiated but metabolically less active cells, like lymphocytes or nucleated erythrocytes, the inactivated nuclei of the differentiated cells undergo a marked activation process.

The formation of reconstituted cells by the fusion of the nucleus of one cell type with the enucleated cytoplasm of another is a further, and very important development of somatic cell fusion. Cells cultured as usual and adhering as monolayers to glass or plastic slides are exposed to cytochalasin B—a metabolite produced by the fungus *Helminthosporium dematoideum*; in most cases the nuclei are extruded into the tips of long protrusions. Centrifugation results in breaking off these protrusions so that the cells with the cytoplasm only remain attached to the glass slides and when returned to the culture medium, behave as enucleated cells. Nuclei from another cell strain, obtained by the above method, are then fused with these enucleated cells. For example, human lymphocyte nuclei have been introduced into human tumour cytoplasms in this manner. These cells present very interesting material for the study of gene regulation, nucleocytoplasmic interactions and cell differentiation.

(v) *Somatic cell hybridization, through protoplast culture*: when successful, has important implications in plant breeding, particularly in overcoming cross-incompatibility barriers. A further application may be in the possible transfer of nitrogen-fixing property from leguminous plants to non-leguminous ones. The earliest successful attempt of plant cell fusion was by Carlson who obtained a fertile hybrid between the otherwise sexually incompatible *Nicotiana langsdorffi* × *N glauca*. Later somatic hybrids have been obtained between other solanaceous genera. More exotic combinations, like fusion of chick erythrocytes and plant cells, have not led to later differentiation. The first step in protoplast fusion is its isolation, either through mechanical means or through the use of pectinase and cellulase to remove the middle lamella and cell wall respectively. In order to maintain osmotic stability plasmolyticum or osmolyticum is used. Such isolated protoplasts have been obtained from several plant species. Cells from two separate taxa then are induced to fuse in the presence of polyethylene glycol, leading to aggregation and formation of heterokaryons.

Plant protoplast culture has assumed importance due to the ability of the protoplast originating from any plant to regenerate its own wall within 48 hours and to grow like a normal cell and ultimately generate the entire plant.

# SECTION II
# CHROMOSOME MORPHOLOGY

CHAPTER II : 1

# CHROMOSOME STRUCTURE

THE detailed morphology of the chromosome varies from cell to cell according to the divisional stage it is in. However, it has been accepted as an individual entity, which, despite minor variations due to various factors, retains its individuality throughout the life of the cell.

## Theories of chromosome morphology

The structural details of the *chromosome* have been the subject of research for a long time, particularly due to the fact that it is directly involved in the transmission of hereditary characters. Finer details have been identified with the progressive refinements in instrumentation and methodology so that the earlier concepts are now principally of historical interest.

Some of the older ideas regarding chromosome structure are:

(a) The *alveolar* theory suggests that the chromosome is single throughout the divisional cycle, except at metaphase, when it appears double. During interphase, the chromosomes develop lateral anastomoses and *alveoles* to form a reticulum, which again breaks down at the onset of division.

(b) The *chromomere* theory considers a chromosome to consist of a number of chromatic granules or chromomeres attached together by a thinner achromatic thread. Another concept is that a chromomere is the crest of a wavy chromosome or a twist in the chromosomal spiral. That they are not artefacts can be proved by the fact that if a chromosome is stretched to its fullest extent under a micromanipulator the chromomeres retain their identity and the constancy in their number and position persists through generations of recombination.

(c) The *chromonema* theory suggests that the chromosome is a continuous structure, undergoing spiralisation during the divisional cycle.

A combination of the second and third concepts is advocated by later workers in which the chromomeres are regarded as a part of the chromonema thread which shows a reaction different from the interchromomeric regions. Chromomeres are then defined as the active regions on the chromonema where nucleoprotein synthesis takes place during the divisional cycle so that they resemble stained beads on a string.

The main controversies involving the structure of the chromosome are two-fold—(i) whether it is composed of one or more longitudinal threads, and (ii) at which stage does the chromosomal thread divide during the divisional cycle. There are three major ideas:

(a) The proponents of the *single thread* theory suggest that each chromosome undergoes division at interphase, forming two identical daughter threads, which move to opposite poles at anaphase. Thus, except at the prophase and metaphase stages, the chromosome has a single thread.

(b) In the *double thread* concept the chromosome is regarded to divide one cycle in advance at prometaphase so that the metaphase chromosome is 4-partite, becoming 2-partite at anaphase. Treatment with nitric acid vapour and infrared studies have shown the double nature of the anaphase chromosome, providing evidences in favour of this concept.

(c) The *quadruple* thread theory holds that the chromosome is 4-partite at anaphase. On splitting at prometaphase, the chromosome becomes 8-partite.

Later developments, showing the DNA thread to be the integral part of the chromosome, have outdated these concepts. The synthesis of DNA and the consequent duplication of the thread have been shown to take place during interphase. The basic morphologic unit is a fibre approximately 100Å thick formed by the association of DNA with histones.

Taylor (JH 1967 In *Molecular Genetics* **2**:95 Academic) suggested that the anaphase chromosome is monofilamentous with double helix DNA, being the unit of duplication and segregation.

Regarding the presence of DNA molecule at a uninemic or polynemic level, suggestions have been put forward in favour of both concepts. The most compelling evidence of polynemy has been the variation of DNA content of allied species having otherwise similar number, morphology and size of chromosomes (see Callan HG 1972 *Proc R Soc* **181 B:** 19).

Light and electron microscopic studies, enzymic digestion and *in vivo* observations support the concept of the multifilamentous nature of the chromosome. Artificially produced chromosome breakage and enzymic digestion show that the bipartite nature of the anaphase chromosome is not an artefact. The metaphase chromosome is seen to be multifibrillar with a structural unit of a 125Å thick filament, paired successively with units of equal thickness to make up a whole chromosome.

hemisubchromatid
subchromatid<
chromatid<
Chromosome< etc.

It was assumed initially that each half-chromatid contained a long DNA molecule, though this concept of binemy could not explain genetic recombination. A model to be acceptable must be able to explain the different facets of chromosome behaviour.

The concept of *uninemy*, that is, each chromosome contains a single DNA helix running from one end to the other, gradually became established with evidences accumulating in its favour. Britten RJ and Kohne DE (*Science* **161**: 529 1968) showed that DNA in eukaryote chromosome could be grouped into three categories, depending upon the degree of repetition of certain base sequences. The very highly repetitive types, with up to a million or more copies of a similar sequence, constituted about 2 to 10% of the total DNA. The moderately repetitive DNA with sequences copied between hundreds to thousands of times formed 20-40% of the total DNA. In addition, there was a third type of genes which was present only in a single copy as in sperm DNA (Laird CD 1971 *Chromosoma* **32:** 375). Their presence could not possibly be explained by the multinemic concept of chromosomes.

Isolation of classes of DNA molecule with very high molecular weight in *Drosophila* and yeast also indicated that one chromosome contained only one DNA molecule.

The development of the interchromatid exchange technique showed definitely that exchanges between sister chromatids involve the single DNA thread, which has been detected through the advancements in methodology (Latt SA 1973 *PNAS* USA **76**: 3395; Perry P and Wolff S 1974 *Nature* **251**: 156).

CHAPTER II: 2

# EUCHROMATIN AND HETEROCHROMATIN

## Definition

When a chromosome in a higher organism is observed during the mitotic cycle under light microscope, with conventional fixation and staining procedures, different parts of it show different condensation cycles. Interphase chromosomes cannot be distinguished individually. The interphase nucleus is seen to contain dispersed chromosome material and certain highly condensed bodies or *chromocentres*. The metaphase chromosome, on the other hand, has brightly stained condensed segments or arms with a few regions, usually specific in number and position, which are unstained. The term *heterochromatin* was originally introduced by Heitz in 1925-1928 to denote those chromosome regions which remained in a highly condensed state throughout the divisional cycle and did not unravel in telophase like the rest of the chromosome; the parts that underwent normal uncoiling in telophase were termed *euchromatin*. He adopted the term from *heterochromosome* or sex chromosome since in some plants, the sex chromosomes are heterochromatic in nature. His definition gave the first concrete shape to the concept of heterochromatin.

Since then a number of unique properties have been attributed to heterochromatin, including amongst others, genetic inertness, position effect, temporary genetic inactivity, absence of template activity *in vitro*, late replication in the synthetic phase, a high content of redundant DNA, specific breakage with maleic hydrazide, abnormal mitotic coiling after cold treatment and specific staining with certain fluorochrome dyes. However these properties and others described by earlier authors, are not common to all heterochromatin. Heterochromatin, as it is known now, is a state showing properties different from euchromatin—the regions exhibiting the regular staining and condensation cycle of the chromosome. This definition includes the regions which remain condensed throughout the divisional cycle, as observed by Heitz; which remain decondensed throughout, e.g., the secondary constriction regions and also those which exhibit allocycly, as suggested by Darlington (CD 1965 *Cytology* Churchill London).

Some of the earlier definitions of heterochromatin include:

(i) A region which responds to staining in a way different from the rest of the chromosome; it arises through the tandem duplication of a single gene so that it is composed of a set of similar genes, differing from others in their nucleic acid content (Pontecorvo).

(ii) A region which fails to maintain the maximum nucleic acid cycle and cannot compete with the rest of the chromosome in nucleic acid synthesis; therefore it exhibits allocycly, being brightly stained in interphase and unstained at metaphase, as opposed to euchromatin (Darlington and La Cour).

(iii) A whole chromosome or a part, located mainly close to nuclear or nucleolar membrane, which exhibits differences in its nucleic acid cycle or content with the rest of the chromosome(s) (Vanderlyn).

## Function

Conceptions regarding the function of heterochromatin were also variable, as for example:

(i) Darlington suggested that these regions gave a selective value to plants but were not indispensible.

(ii) Mather postulated the presence of two types of genes: (a) the major oligogenes, which were inherited according to the Mendelian ratio, produced major phenotypic changes and were responsible for qualitative characters; obviously they were located in the euchromatic regions and (b) the polygenes which produced small, similar and additive effects and controlled quantitative inheritance; they were located in the heterochromatic regions.

(iii) Vanderlyn, on the basis of his observation that heterochromatic regions are localised near the nuclear or nucleolar membranes, regarded these regions as "liaison" chromatin, through which nucleo-cytoplasmic transfer of RNA takes place.

(iv) It was regarded to represent repressed portion of the genome as indicated by the preferential heterochromatinisation of one chromosome in the mammalian female.

## Types

Regions showing heterochromatic properties can be broadly classified into two groups: constitutive and facultative. *Constitutive* heterochromatin is present in the same position on two homologous chromosomes and is inherited. *Facultative* heterochromatin develops later during the course of development of an organism. Constitutive heterochromatin in both paternal and maternal chromosomes responds directly and similarly to development as it is inherited. Facultative heterochromatin is related to development as well, but it occurs in one of two genetically alike complements so that the two chromosomes behave differently. It may, in some cases, have an extrachromosomal control. Its significance is due to its capacity for shutting off normal gene function for relatively longer or shorter periods during the lifetime of an organism. The terms, alpha and beta heterochromatin, have also been used to differentiate two types of heterochromatin. α-heterochromatin is compact and brightly stained, positively heteropycnotic and not susceptible to external agencies and is therefore important for phylogeny such as, the Y chromosome in man while β-heterochromatin is diffuse and lightly stained, negatively heteropycnotic, variable and can at times be converted completely into euchromatin such as the X chromosome at certain phases. The former has possibly fewer genes while the latter has the property of shutting off gene action in the areas concerned.

The earlier concepts of heterochromatin were mainly based on observations of the different heterochromatic segments and therefore the properties attributed to these segments differed as well.

### Constitutive heterochromatin

Constitutive heterochromatin, in most cases, has been found to stain intensely with the typical dyes used for chromosomes like Feulgen, following a cycle different from that of euchromatin. It tends to have a constant position on homologous chromosomes.

*Within a chromosome*, it has been found to be located at specific regions, such as:

(a) *Centromeric*: It occurs on the two sides of the centromere as a destained gap, the primary constriction, during metaphase. From telophase onwards it takes up stain and is observed as brightly stained *prochromosome* during interphase. It apparently plays a role in the movement of the chromosome along the spindle. It may influence chromosome orientation determining which member of a pair has to go to the sex cell. In the somatic cell of *Drosophila melanogaster*, the heterochromatin occurs in large bands at the centromere of each autosome and the Y sex chromosome is entirely heterochromatic. In the salivary gland chromosomes, however, which are about 100 times longer, the amount of heterochromatin is about 1/20th of that expected from the autosomal picture. The Y chromosome and the centromeric heterochromatin fuse into a common *chromocentre*, thus providing an example of tissue to tissue variation in heterochromatin content.

(b) *Telomeric* regions are found to have a different staining cycle than the rest of the chromosome arm in some plants. They may act as protective covering for the inner active genes.

(c) *Nucleolus-organising regions* are observed on specific chromosomes as destained gaps known as secondary constriction regions. They are seen associated with the nucleolus at telophase and do not take up stain at any stage of the divisional cycle.

(d) *Intercalary heterochromatin* is observed in the form of bands between euchromatic segments. They are brought out clearly through different treatments, in the form of fluorescent banding patterns after staining with fluorochromes like quinacrine mustard, which serve as identifying criteria in mammalian chromosomes. The bands in the salivary gland chromosomes of *Drosophila melanogaster*, other than chromocentre, are also examples of intercalary heterochromatin.

*Entire chromosomes* may exhibit heterochromatic behaviour, including: (i) sex chromosomes in some plants, and (ii) supernumerary or accessory chromosomes. They occur in certain plant populations, as for example, the B chromosomes in rye. They are usually present in tissues not involved in transmission, occurring in variable numbers in addition to the normal somatic complement. They apparently confer a selective value on the population concerned.

## Facultative heterochromatin

Facultative heterochromatin is seen to develop during the development of an organism.

An example where an entire chromosome becomes heterochromatic in the course of development is provided by one of the X chromosomes in a mammalian female. In a normal human male, the sex chromosomes are XY and in a normal female XX. The X chromosome in the male and one of the X chromosomes in the female remain euchromatic throughout the life cycle. However, the other X in the female becomes heterochromatic in the course of development from the zygote to the embryo. This heterochromatic X or "hot X" takes up distinctive stain and forms the basis of sex identification in sex chromosome abnormalities. The function of the heterochromatin in the hot X of a mammalian female is apparently to equate the number of functional X in the male (X+inert Y) with that in the female (X+hot X). As a result the genes of the hot X are repressed and only one X remains functional, compensating for the extra dosage of genes in XX female. In abnormal individuals with more than 2X chromosomes, all X's, *except one*, become heterochromatic. In tumours studied from human females, the cells have the same X chromosome composition throughout, thus showing that tumours are probably derived from single cells.

Example in which an entire genome becomes heterochromatic during development is shown by the mealy bug. During the development of the male insect, the entire chromosome complement that it has inherited from its paternal parent becomes heterochromatic while the complement inherited from the maternal parent remains euchromatic in nature. Thus the male bug can contribute only one kind of gamete—that containing its maternal chromosome complement. In a female offspring, this complement becomes heterochromatised and lost before reaching the stage of gametogenesis.

The heterochromatised X chromosome of a mammalian female and the paternal chromosome complement in a male mealy bug are suitable examples of facultative heterochromatin formed from normal euchromatin during development.

During polyploidisation as well in plants, the extra genome contributed through duplication undergoes repression to a certain extent so as to permit only the number of genes compatible with the environment to function.

## Properties

Different properties have been exhibited by the different forms of heterochromatin, unique for the type present in one cell or another. All these properties are not associated with any particular type of heterochromatin, nor do all forms of heterochromatin show similar behaviour. With a gradual understanding of the chromosome chemistry, optically identified characteristics have been correlated with the molecular structure.

GENETIC INERTNESS: Earlier authors considered all heterochromatin to be genetically inert since addition or loss of heterochromatic segments did not have an appreciable phenotypic effect and these segments are not involved in chiasma formation or in the occurrence of spontaneous mutations. However, the concept of complete inertness was modified when Müller demonstrated the presence of the "bobbed eye" gene on the Y chromosome of *Drosophila*, which was heterochromatic and therefore presumed to be inert. Similarly Gates suggested the location of the gene for hairy earlobes in man on the supposedly inert Y chromosome. Thus they regarded the heterochromatic regions to be composed of not degenerate genes but rather of nongenic material derived from a few active genes. The proportion of genes on heterochromatic and euchromatic regions in salivary gland chromosomes however was found to be same on a length-for-length basis. In tomato, a gene has been localised on a heterochromatic segment. Crossing-over, however, has been found never to involve heterochromatic segments, except for mitotic exchanges in *Drosophila*. Apparently, the concept of genetic inertness for heterochromatin is not of universal application. It is not feasible to equate heterochromatin (or condensed chromatin) with inactive chromatin and euchromatin (or dispersed chromatin) with active chromatin, since nuclei can be dispersed with chelating agents without changing the relative proportions of active or inactive chromatin. The large X chromosomes of *Microtus agrestis* are inactive in interphase even in those cells where they are dispersed, that is, euchromatic. Therefore chromatin does not have to be condensed to be inactive though generally inactive chromatin is in a highly condensed form. The control of activity is evidently at the level of the basic deoxynucleohistone fibre. Heterochromatin is, according to Ris H and Kubai DF (1970 *Ann Rev Genet* 4 263), a term to be used only for the morphologically condensed state of chromatin, without reference to its activity.

POSITION EFFECT: A property attributed to heterochromatin was that of heterochromatinisation of adjoining euchromatic segments. If by design or accident, a heterochromatic segment is translocated next to an euchromatic one, the activity of the genes on the latter is heterochromatised, a phenomenon known as *position effect*. As a result, the phenotypic effect controlled by that particular euchromatic gene shows variegation. However, euchromatic genes have been observed to exert a similar effect on heterochromatic ones. For example, the addition of an extra Y chromosome has the opposite effect, of depressing position effect. The position effect shown by the addition of a hetero- or euchromatic gene possibly is caused by a disturbance in the preconditioning of the contiguous gene during development.

TEMPORARY GENETIC INACTIVITY observed in certain chromosome segments may render them facultatively heterochromatic and is of significance in explaining differentiation. Genes were observed to lose their functional abilities for relatively brief periods. The chromosome structure in embryos was different from that in older functioning tissues. In certain insects such as coccids a given chromosome set becomes heterochromatic during embryonic development and at a later stage reverts to the euchromatic state.

ABSENCE OF TEMPLATE ACTIVITY IN VITRO: Heterochromatic regions are unable to synthesise RNA *in vitro*. This property has been attributed to the fact that chromatin fibres in these regions are more tightly packed than in euchromatin. The histone contents of both types of chromatin are similar. Therefore, it has been suggested that the polyanionic components which act as de-repressors for histone are absent or ineffective in the heterochromatin. It is supported by the fact that these components are less in amount in heterochromatic segments.

LATE OR EARLY REPLICATION IN THE SYNTHETIC PHASE: When tritiated thymidine is incorporated into cells of a tissue, the heterochromatic segments are seen to replicate their DNA later and sometimes earlier than the euchromatic ones. This can be studied in materials with large blocks of heterochromatic regions, which are brightly Feulgen positive at interphase, so that the autoradiographic pictures can be compared with the morphological one. When the same region, in a different tissue or at a different stage of development, is euchromatic in behaviour, its DNA replication cycle follows the normal routine. The timing of DNA replication is affected by differentiation. There are organisms where the time of DNA synthesis of a given chromosome changes during development. This phenomenon is connected with transition from euchromatin to heterochromatin.

REPETITIVE DNA: A high content of repetitive and possibly repressed DNA has been found in particular heterochromatic regions in certain cases. For example, extra copies of ribosomal cistrons have been observed in the nucleolus—associated heterochromatin of *Acheta* and other insect species, together with a predominant amount of other DNA sequences of no known function. This phenomenon explains the low phenotypic effect following loss or addition of heterochromatic segments.

BREAKAGE WITH DIFFERENT AGENTS, INCLUDING MALEIC HYDRAZIDE: Heterochromatic regions are easily broken by external agents like X-ray and radiomimetic chemicals. However, whether they are more susceptible or whether breaks at these regions are more likely to survive due to non-involvement of essential genes is the question.

ABNORMAL MITOTIC COILING AFTER COLD TREATMENT: Cold treatment results in the formation of destained gaps, referred to earlier as "nucleic acid starved" areas, due presumably to the loss of nucleic acid from these regions. However, later workers have suggested that these regions should not be included under heterochromatin. Spectrophotometric measurements show that the amount of DNA per unit area is 2 or 3 times higher in the heterochromatin than in the euchromatin. Also autoradiography has revealed the occurrence of such cold-induced "starved" regions in chromosomes which had completed their DNA synthesis before the cold treatment started. These evidences indicate that such regions are, therefore, different from heterochromatin in the conventional properties.

SPECIFIC STAINING WITH CERTAIN FLUOROCHROME DYES: Different regions of chromosomes differ in the intensity of fluorescence seen when they are treated with fluorochrome dyes like quinacrine mustard. They give bands of different intensities, which may be used to identify the chromosomes. The high proportion of repetitive DNA in these regions is a related phenomenon.

ALLOCYCLY OR HETEROPYCNOSITY was the first property attributed to heterochromatin. It was identified on the basis of its maintaining a condensed state throughout the interphase when the remainder of the nuclear chromatin was extending to the euchromatic state. This property is, however, not universal, since many forms do not show complete allocycly. It is exhibited principally by the heterochromatin on the two sides of the centromere.

## Banding

The techniques for *Q*, *G*, *R*, *O* and *C*-banding, developed in recent years, present the single DNA double helix and its associated proteins within a single chromatid as tightly coiled and folded in the metaphase chromosome. Heterochromatic regions remain in this compacted state in interphase, are tightly bound by non-histone proteins, undergo a late DNA-replication and appear as positively staining bands in metaphase with *Q* and *G* and *O* techniques and negatively stained *R*-bands (Evans HJ 1973 *Br Med Bull* **29**: 196). The *C*-band regions correspond with the centromeric type of heterochromatin, which may be located, as in certain plants, in regions other than the centromere also. The *G*-bands and *O*-bands correlate with the intercalary type of heterochromatin and with the *Q*-bands as well. The heterochromatic regions possibly are folded in a pattern different from the euchromatic regions (Comings 1974).

The techniques developed distinguish basically between the three major states of chromatin: centromeric and intercalary heterochromatin and euchromatin (see Table II: 2.1)

**TABLE II: 2.1**

**Comparison of different types of chromatin**

| | *Constitutive heterochromatin* | | *Euchromatin* |
|---|---|---|---|
| | *Centromeric* | *Intercalary* | |
| *Location* | | | |
| i) in bands | C bands | $G_1$ bands | R-bands |
| ii) on chromosome segment | Centromere | Arms | Arms |
| iii) in pachytene chromosomes | Centromeric chromomere | Intercalary chromomere | Interchromomeric region |
| *Interphase* | Condensed | Condensed | Dispersed |
| DNA replication | Late S | Late S | Early S |
| Genetic activity | Inactive | Relatively inactive | Usually active |
| Content of satellite DNA | +++ | ± | ± |
| Repetitious DNA content | High | Moderate and unique | Moderate and unique |
| Base composition | GC-rich, neutral or AT-rich depending on satellite DNA | Slightly AT-rich | Slightly GC-rich |
| DNA methylation (methylcytosine) | +++ | ± | ++ |

## Conclusions

Heterochromatin was originally defined by its expression in the phenotype of the chromosome (s) and it is still today recognised primarily by the changes that occur at this level. Since then increasingly detailed studies of heterochromatic regions, particularly the heterochromatic X chromosome of the mammalian female, have revealed that heterochromatin represents repressed portions of the genome, that it offers direct cytological evidence of the degree of nuclear differentiation and of the regulation of gene expression in higher organisms. The amount of histone present in both types of chromatin is the same. The chromatin fibres are also identical, as observed from electron microscopical studies except that fibres in heterochromatin are more tightly folded, which may account for some of its metabolic peculiarities. What transpires at the gene or molecular level, when the heterochromatin becomes visible under the microscope, is mainly unknown.

DNA molecules within native euchromatin complexes are extended microfibrils of 100Å diameter and differ from the DNA molecules existing in a highly condensed state within native heterochromatin complexes both in their secondary and tertiary physical conformations and in the magnitude of their specific activity as templates for RNA synthesis or early DNA synthesis. These differences are lost after isolation. Therefore, they are possibly not intrinsic to DNA but are stabilised by other macromolecules associated with the DNA within heterochromatin. The activity of euchromatin has been attributed to an excess of nuclear polyanions in them which de-repress DNA molecules for template activity in RNA synthesis by reducing the interaction between DNA templates and histone repressors.

## Evolution

An interesting theory was proposed for the evolution of heterochromatin from the available data on the base composition of early and late replicating DNA, centromeric and intercalary heterochromatin and essential and non-essential DNA, as observed in mammalian chromosomes (Comings DE 1972 *Exp Cell Res* **71**: 106). A mammalian chromosome is composed of genetically active euchromatic regions interspersed with heterochromatin and satellite DNA. In the first part of the synthetic phase, molecules rich in G-C are synthesised and this euchromatic DNA is localised in the long arms of chromosomes. During the last part of the S-phase, however, heterochromatic satellite DNA, rich in A-T, appears and occupies the regions around the centromeres and short arms of the chromosomes. In the proposed model, it has been suggested that the primitive early genome was composed mainly of active essential genes, which were relatively G-C and methylated cytosine (mC) rich. Since methylated cytosine can be converted to thymine through the action of aminohydrolase, a mutation from mC to T is possible in nature. With continued gene duplication and increase in DNA content, some of the genes mutate to non-functioning status. Thus relatively non-essential DNA is accumulated which separates the essential genes. Further increase in DNA leads to further duplication of such genes. One mC to T mutation in these non-essential genes is preserved due to lack of selection pressure, and may through continued duplication, lead to the A-T rich heterochromatic DNA. However, the fallacy underlying this theory is that it presumes that heterochromatic DNA has little functional role, a presumption which may not be valid. Constitutive heterochromatin has been regarded to have a distinct role in evolution. Groups of organisms possessing relatively more heterochromatin may be expected to be more successful in speciation, providing a variety of different karyotypes. Such an increased number of viable chromosomal rearrangement favours reproductive isolation—as a preliminary for speciation. When new sources of constitutive heterochromatin arise through saltatary replication, they may induce an old pecies, which has reached a dead end in evolution, to give ise to new forms.

CHAPTER II: 3

# MODEL OF CHROMATIN STRUCTURE

## Chromomere

In the meiotic prophase and early mitotic prophase, the chromonema thread is seen to have dense thickened areas with thinner regions in between, giving the appearance of a string of beads. These regions were known as chromomeres and their position, and size as well, are constant to a certain extent. They have been regarded as genetically significant regions of the chromosomes, though this idea has been a controversial one. The different concepts regarding their nature suggest: (a) that they are condensations of nucleoprotein material, and (b) that they are regions of superposed coils. The latter idea had received some support from electron microscopic observations.

In polytene chromosomes identical chromomeres on parallel chromatids give rise to the characteristic bands. Both chromomeres and interchromomeric regions have been found to contain DNA, consisting of 250Å fibres. In meiotic chromosomes, the same kind of DNA is present in a tightly coiled condition in the chromomere but is straight and parallel to the chromosome axis in interchromomeric regions.

Chromomeres have been regarded as the units of replication, transcription, mutation and recombination. Cytogenetic data localising mutations in specific chromomeres and the appearance of puffs and loops—regarded as structural manifestation of gene activity—have been cited as evidences for equating chromomeres with units of gene expression. However, this concept is still open to question. At most, the chromomere can best be considered as a unit of functional coordination.

### Model of chromatin structure

The semiconservative nature of replication and the Mendelian ratio followed by mutational events support the view that each chromatid is a *single* DNA double helix, complexed with histone and non-histone proteins. The DNA-histone ratio is 1 while non-histone/DNA ranges from 0.2 to 3.0, being directly proportional to the genetic activity of the tissue. Electron microscopy and x-ray diffraction studies have shown that the chromosome structure is beaded in appearance, the beads being termed as *nucleosomes* or the smallest units of a chromosome. DNA is supercoiled into a fibre 100 Å wide with a distance of 120 Å between coils. This fibre is further coiled into the 200-300 Å wide chromatin. Nucleosomes are visualised as 10 nm in diameter and connected with each other by a chain of DNA (see Fig. II: 3.1).

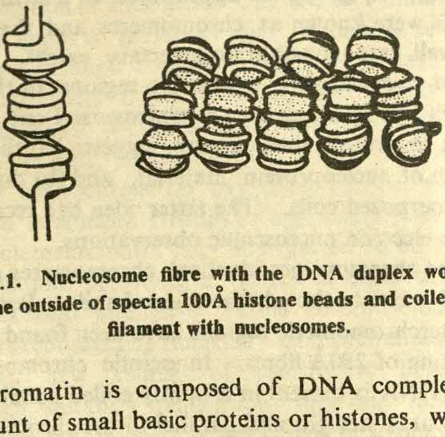

**Fig. II : 3.1. Nucleosome fibre with the DNA duplex wound around the outside of special 100Å histone beads and coiled nuclear filament with nucleosomes.**

The chromatin is composed of DNA complexed with an equal amount of small basic proteins or histones, which may be arginine or lysine-rich. Five histone fractions enter into the composition of nucleosomes. Four of them, H2A, H2B, H3 and H4 form an octamer while H1 is associated as a linker. Histones 3 and 4 are almost completely conserved during evolution. A DNA molecule of 200 base pairs coils round the octamer on the outside. The entire structure has the shape of a flattened disc 11 nm in diameter and 5.7 nm in height as observed in an electron micrograph. Taking the average mol wt of a histone to be 63,000, an octamer has a mass of 105 daltons. Of the DNA molecule, 140 base pairs enter into the coiling of the octamers and a variable number (15 to 180 base pairs) goes to form the linkers between different nucleosomes. Only one H1 molecule is associated with the linker, which connects one nucleosome with another. The H-1 histone fraction is present only once for 200 base pairs of DNA. It varies considerably between species and even between tissues of the same organism. The core DNA of 140 base pairs is highly conserved. Neighbouring nucleosomes can be folded into a 20-30 nm fibre (*ultimate fibril*). A solenoidal structure is thus produced having 6 nucleosomes per turn, which is stabilised by interaction between the H-1 molecules running through the core. This 20 to 30 nm fibre can be observed in metaphase chromosomes and interphase nucleus and probably represents the natural conformation of transcriptionally inactive chromatin. Packing of DNA in a chain of nucleosomes is about 5 to 7 times more compact than free DNA. In the solenoid structure it is packed about 40-fold. This compaction is however much lower than in metaphase chromosomes (5000 to 10,000 fold). However, the nucleosomes represent *repeats* when considered on the basis of the protein structure. Thus the DNA in the nucleosome configuration consists of a core of 140 base pairs attached at intervals of 10 by deoxyribonuclease I and linker DNA with base pairs varying in units of 10, half of it being susceptible to cleavage by micrococcal nuclease. At the free end also, the DNA thread can be trimmed rapidly by micrococcal nuclease. Repeated DNA is regarded to present a similar picture (Fig. II.3.2).

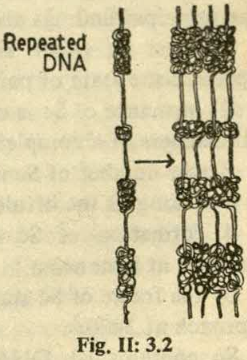

Fig. II: 3.2

The different banding techniques, used to identify chromosome segments, either bind to DNA by intercalation between base pairs (fluorochromes); or by attachment to the phosphate groups, or to the outside portion of base pairs without intercalation. This diversity in the modes of attachment of dyes may be related to the binding of the non-histone proteins to the DNA in the different types of chromatin.

Later workers have suggested that the histone H4 may be responsible for the coiling and uncoiling of chromosomes at metaphase. They were of the opinion that the ultimate fibre coils into loops, which further coil to give the appearance of chromosomes. The process is reversible.

The nucleosomes, however, are now regarded as too small to be in the magnitude of an actual gene. The circular SV40 DNA has 5224 base pairs with a nucleosome size of 187. The number of nucleosomes present is 22 while the linker DNA (23% of the total), varies in size. The number of genes on the SV40 DNA is, on the other hand, seven, each gene thus containing about 800 base pairs. Apparently, nucleosomes may be much smaller than actual genes. A nucleosome, therefore should be considered as *the structural unit* of a chromosome but *not* tne genetical one.

SYNAPTONEMAL COMPLEXES (Sc) are structures occurring in gametocyte nuclei. At the beginning of prophase I they may form part of the paired chromosomes and accompany their movement from joining to synapsis. Each complex has two main units of lateral elements (LE) with a layer of material in between apparently formed by LE. These have been mainly studied in spermatocytes and oocytes under electron microscopy and the studies have helped in clarifying morphology of chromosomes. The principal findings are:

1) most Sc occur simultaneously with the bivalents and represent the state of pairing of the latter.

2) structure of Sc is constant for a species and is same for all members of a complement.

3) the number of Sc is the same as the haploid one and each Sc is as long as the bivalent.

4) formation of Sc depends on a small amount of DNA synthesis at leptonema.

5) the frame of Sc starts forming when the two homologues approach at 3000Å.

Sc contains (i) DNA and basic protein; (ii) only protein binding to single stranded DNA or double DNA with gaps or and (iii) polynucleotide ligase.

The points needed to be clarified about Sc are: (i) its importance in genetic transfer; (ii) whether the space in between contains DNA.

## CHAPTER II: 4

# CENTROMERE AND SPINDLE MECHANISM

**Definition:** The *centromere* or the *kinetochore* is a specialised region of the chromosome associated with its movement along the spindle to the two poles during anaphase. It may be *localised* on a particular part of the chromosome, the primary constriction region, or it may be *diffuse*, in which the entire chromosome shows the property of spindle attachment.

**Localised morphology:** The localised centromere appears basically as a small destained gap during metaphase and gives the chromosome its shape. This appearance is due to the presence of two blocks of heterochromatin at the two sides of the centromeric apparatus, which exhibit allocycly. They are destained at metaphase and stained at interphase to form the brightly stained bodies or *prochromosomes* at the interphase nucleus.

The detailed structure of the centromeric apparatus has been gradually clarified through improvements in chromosome methodology. The optimum stage for study is pachytene when it is the largest and the best materials are plants with long chromosomes like *Hyacinthus* and *Trillium*. The use of pretreatment chemicals, particularly of oxyquinolene, and subsequent application of pressure result in the contraction of chromosome arms, stretching out the centromere on the solidified plasma.

Based on the relative positions of the centromere, chromosomes are described as metacentric, submetacentric, subtelocentric and telocentric. In insects, when both arms of a metacentric chromosome show a similar capacity of condensation, these are referred to as *monophasic*. Metacentrics with one heterochromatic and the other euchromatic arms are *diphasic* and this differential behaviour of the two arms can only be studied following treatment with chemicals like colchicine to bring out cryptic heterochromatin.

MORPHOLOGICAL CONCEPTS: Till McClintock in 1933 first demonstrated the centromere to have a distinct structure, it was earlier regarded as a genetically inert gap in the chromosome body. Subsequent workers regarded it as an ordinary chromomere, a temporarily modified chromomere, a modified portion of the genonema or chromonema adapted for a specific function and a set of spindle spherules attached to the genonema, which extends throughout the length of the chromosome. Darlington regarded it as a multiple gene composed of similar and supplementary parts like polygenes, a concept which explained the phenomena of misdivision and the survival of telocentrics.

The later theories about the detailed structure of the centromere evolved with the evolution of the pretreatment and squash schedules which permitted its clarification.

(i) The first concept, developed by Tjio and Levan in 1950 after pretreatment with oxyquinolene, regarded the metaphase centromere to have a quadruple nature. Within an unsplit chromosome, the centromere consists of three regions: (a) two large granules or chromomeres lying side by side; (b) an —o—o— interchromomeric thread joining them, and (c) two threads each joining one chromomere with the corresponding chromosome arm. The granules and the threads stain positively with Feulgen. With the splitting of the chromosome, the chromomeres and the threads divide longitudinally as well, to give four identical chromomeres arranged in a parallelogram, having chromatic threads connecting them longitudinally with each other and with the chromosome arms. The chromomeres form mirror images of each other.

(ii) In a modified version (Lima-de-Faria) small chromomeres in the interchromomeric zone as well as on the threads connecting the larger chromomeres with the chromosome arms were seen and the two halves of the centromere were identical in morphology and function.

(iii) Observations from the pachytene stage of the iampbrush chromosomes of *Triturus* suggested that the large outer chromomeres were made up of two or more small chromomeres, located very close to each other. Each of these chromomeres was again longitudinally double.

This morphological structure of the centromeric apparatus is able to explain the different behavioural patterns associated with the centromere. During normal division, the centromeric chromomeres and threads divide lengthwise so that an identical replica is transmitted to each daughter chromosome. However, under certain conditions, transverse division has been observed, referred to as *misdivision* or bursting of the centromere (Darlington). The resultant daughter chromosomes have terminal centromeres. There are three possible fates for such telocentric chromosomes: (a) the telocentric chromosome may continue to exist as a member of the normal complement, as for example in *Phleum echinatum* and *Pterotheca falconeri*. A high number of telocentrics has been recorded in *Oxalis dispar* (2n=12) with 7 and *Tradescantia macrantha* (2n=24) with 22 telocentrics. These plants possibly have a limited evolutionary significance. (b) The telocentric chromosome divides longitudinally and one arm rotates through 180° to form an *isochromosome*, which has both arms identical and completely homologous. During meiosis, the two arms pair and on terminalisation of the chiasma, form a ring. Isochromosomes have been observed in *Nicandra* and isochromosome X is associated with certain human aberrations. (c) Occasionally two isofragments arising through misdivision, may fuse at the centromere.

The phenomena of misdivision and the origin of isochromosomes can be explained by the models of centromeric apparatus detailed above. Since the centromeric granules are mirror images of each other, misdivision causes telocentric fragments. In the latter, even though one or more such granules may be absent, the remaining chromomeres are sufficient to carry on the function of spindle attachment. On the formation of isochromosomes, the complete set of chromomeres is again obtained, restoring the centromeric apparatus. Centric fission and fusion are also easily explained according to this structure.

ULTRASTRUCTURE: Electron microscopic studies have given further details of the structure of the localised centromere at the ultrastructural level. It appears as a bundle of microtubules originating at a specialised region and connecting the chromosome with the spindle.

Various types of connections are seen between microtubules and chromatin:

(i) The microtubules may terminate next to the twisted chromosome fibrils without any special structure, as in *Amoeba*.

(ii) The region where the microtubules terminate is denser than the rest of the chromatin, as in sea urchin.

(iii) In certain higher plants like *Lilium* and *Tradescantia*, the microtubules end in a spherical mass of fibrils, less dense than rest of the chromosome, embedded in a cup-shaped indentation of the chromatin.

(iv) In mammals and a moss, *Mnium*, the structure is very complex. The microtubules end in a band or disc 250Å thick, separated from the chromatin by a less dense area. The dense plate seems to contain two or more 250Å fibres that often appear double and may be continuous with chromatin on either side of the kinetochore. These fibres are surrounded by less dense fibrous material. The kinetochore has been compared with a lampbrush chromosome loop suggesting that it is a specialised active region.

The conception of the structure of the localised centromere has evolved from a parallelogram of four chromomeres attached by threads, to several small chromomeres attached by threads to one or more filaments per chromatid forming a dense core, from which microtubules emerge and loop out, given a lampbrush-like effect. The density of the microfibrils may be responsible for the chromomere-like appearance. The filament originates at one point on each chromatid, winds round its surface and re-inserts into a point further along the same chromatid. Two such filaments may be present in each chromatid. Five to seven chromatin fibres, each 230Å in diameter, have been calculated to pass through the centromere. In addition, each chromosome has two disc-shaped sister kinetochore granules, each with a diameter of 0.2$\mu$, attached to 4 to 7 hollow spindle tubules. Thus the two functions of the centromere—of holding the two sister chromatids and binding the spindle fibres—are assigned to two different components. Inoué supports the idea that the centromere is directly involved in the organisation of microtubular subunits into chromosomal spindle filaments. This structure of the localised centromere is also able to explain its associated phenomena. Late replication of the chromatin fibres passing through the centromere holds the sister chromatids together. The latter start separating as soon as the kinetochore chromatin replicates.

SYNAPTONEMAL COMPLEX: In the meiotic chromosomes, on the other hand, a special structure appears during the synapsis of bivalents between paired chromosomes. Known as the *synaptonemal complex*, it is a tripartite body located between the synapsed chromosomes. It is composed of two lateral dense fibres, twisted around each other, with a flanking central ribbon. The lateral arms are joined to the adjacent chromosomes by fibrils of about 10 nm in length. The microfibrillar material arises from the chromosomes themselves whereas the synaptonemal complex is proteinaceous, consisting mainly of scaffold proteins (see chapter II :3 p. 64).

EM *in situ* hybridization shows that centromeres tend to face each other with concomitant labelling of chromatin fibres between adjacent centromeres. It may be that *in vivo* centromere regions tend to interact through homologous DNA sequences and that clusters of mitotic centromeres may coalesce to form typical heterochromatin-containing bodies in interphase nuclei of mouse cells.

DIFFUSE CENTROMERE has been observed in certain special groups of plants, like *Luzula* and several members of Cyperaceae and in certain Hemipteran insects, like *Steatococcus* and *Tamalia*. The kinetochore is not localised on a particular position in the chromosome but the property of spindle attachment is distributed throughout the length of the chromosome. The evidences for the diffuse nature of these chromosomes are threefold:

(a) The chromosomes are rod-shaped during anaphase, showing parallel movement to the poles.

(b) On breaking up these chromosomes with x-ray treatment, short fragments are formed, all of which behave like the normal parent chromosomes. In case of a chromosome with a localised centromere, however, the fragment without the centromere is lost during the divisional cycle.

(c) After fragmentation, during the subsequent meiosis, each fragment pairs with its corresponding segment on the intact chromosome, showing their homology.

In studying the ultrastructure of chromosomes with diffuse centromere, in some cases dense material was arranged in thin layers on either side of the chromosome at metaphase, though no tubules were seen. However, in *Philanus* sp. microtubules penetrate deeply in metaphase chromosomes where they end blindly (see Ris H and Kubai DF 1970 *Ann Rev Genet* **4**: 263).

*Polycentric* chromosomes, that is, with several centromeres have been recorded in *Ascaris*. Similarly, in certain plants, earlier authors had observed a subsidiary centromere along with the normal localised one. The centromere is accepted as evolved through diffuse to polycentric to localised nature. An alternative suggestion of the formation of a polycentric chromosome through fusion of several shorter chromosomes appears less tenable.

On treatment with hydroxylamine, a reagent with preferential effect on cytosine, a large number of breaks is observed in the centromeric regions, indicating these regions to be rich in cytosine.

FUNCTIONS: A function attributed to the centromere is the differentiation of chromosome arms. In certain plants, chromomeres are largest closest to the centromere and decrease progressively towards the two ends.

The principal function of the centromere is attachment of the chromosome to the spindle during cell division, so that the daughter chromosomes may move to the opposite poles. The location of the centromere and the spindle in two different regions, the chromosome and the cytoplasm respectively, renders the organisation of the spindle to be one of the outstanding examples of nucleocytoplasmic cooperation. A possible interpretation is that the spindle substance, which is present in the nucleus, is transferred through the centromere into the cytoplasm and reacts with the spindle precursors there to form the spindle. Birefringence studies have shown that as the chromosomes move from equator to pole, the birefringence of the spindle decreases, suggesting that some substance may be released by the chromosomes to facilitate their movement along the spindle. Belar and Swann, later upheld by Lima-de-Faria, attributed the primary cause of chromosome movement to a chemical activity of the centromere, which secretes a viscous fluid, spreading on the continuous fibre towards the pole, thus allowing a sliding movement of the chromosomes.

The idea of spindle dates back to Flemming but the demonstration of the spindle fibre as an entity was made by Belar and Nebel with the help of a micromanipulator needle. Earlier, the spindle was regarded as a phase difference and the fibrous structure was thought to be an artefact.

FIBRES: In polarised light the spindle is shown to be fibrillar in nature with at least two types of fibres:

(i) the traction or chromosomal fibre, extending from the centromere to the closest pole, and

(ii) the spindle or continuous fibres, running parallel to each other from pole to pole. They constitute the body of the spindle.

An yet a third type of fibre has been visualised, arising between the chromosomes immediately after separation in anaphase—the interchromosomal fibres. Their existence is, however, debatable.

FORCES INVOLVED IN MOVEMENT: Two forces are mainly involved in the poleward movement of the chromosomes after they divide: (i) a pushing force in which the central region between the split chromosomes, after they have divided, elongates, pushing the chromosomes to the opposite poles, and (ii) a pulling force which draws the chromosomes to opposite poles, caused by the shortening of the chromosomal fibres.

The chromosomal fibres are regarded to be transitory in nature by certain workers. They are formed during metaphase and are lost after telophase. The chromosomes are pulled polewards by the shortening of these fibres. Lettré and her group are, however, of the opinion that the chromosomal fibres are permanent entities, attached at one end to the centromere and at the other to an organising centre near the pole. This centre has been visualised outside the nuclear membrane, possibly corresponding to the centrosome in animal cells. The chromosomal fibres from this centre pass through the nuclear membrane, possibly through pores and are attached at the other ends to centromeres. During anaphase, the fibres start winding so that the chromosomes are drawn along the spindle fibres towards the two poles. The chromosomal fibres unwind during interphase and lie loosely within the nucleus. At the onset of of metaphase they wind slightly to orient the chromosomes at the equator and start winding from anaphase onwards.

CHEMICAL NATURE: The chemical nature of the spindle has been studied to a certain extent and reveals the presence of protein, nucleic acid, carbohydrate, lipid, ATPase, free sulphydryl groups and zinc. Electron microscopic studies have also shown several components within the spindle, including a certain number of microtubules associated with the chromosomes.

Spindle fibres are regarded as long chain fibrous proteins, rich in sulphur, extending from pole to pole. They are composed apparently of small protein molecules associated end to end by S-S (disulphide) linkages. The globular protein of the cytoplasm, through possibly the action of the spindle substance secreted by the chromosomes, is converted into the fibrous protein of the spindle. Experimental evidence of this structure was obtained from the fact that on treatment with compounds which break the S—S linkage, like mono- or dimercaptopropanol or digitonin, the spindle is not formed. Colchicine was observed to exert a similar effect. Attempts were made to isolate the spindle from sea urchin eggs in which mitosis was blocked at metaphase with colchicine. The matter thus isolated was an amorphous gel without visible fibres. It was suggested that spindle formation took place in two stages: (i) the formation of a gel through polymerisation by intermolecular S—S bonding; this stage is not affected by colchicine, and (ii) the formation of secondary bonding and orientation of spindle protein into the fibrous system; colchicine inhibits this secondary bonding. ATPase activity appears to be associated with the fibrous component of the spindle.

The relation of the spindle to alignment of chromosomes in metaphase and their movement in anaphase has not yet been explained satisfactorily. It is possible that the spindle fibres are actually attached to the centromere to provide an oriented field for forces inherent in the chromosomes, thus contributing to their movement. Electron microscopy has shown chromosomal fibres to develop from the centromere and to grow towards the corresponding pole. Similarly the loss of acentric fragments during anaphasic separation indicates an actual attachment of chromosomes to the spindle.

MECHANISM INITIATING MOVEMENT: The nature of the mechanism initiating chromosomal movements is a debatable one. Darlington suggests that this mechanism is set off by the division of the centromere. Attraction and repulsion forces are regarded to act between different chromosomes and also between the chromosomes and the pole. In mitosis, the centromere is undivided before metaphase and holds the two chromosomes, which have already divided at interphase, together. When the centromere divides at early anaphase, the chromosomes immediately start their poleward movement. In meiotic metaphase I, on the other hand, the centromere remains functionally single though it divides at the beginning of anaphase I. When the centromeres separate at anaphase II, the chromosomes move apart.

Lima-de-Faria A (1969 In *Handbook of Molecular Cytology*, 277 North Holland) suggested that in both mitosis and meiosis, the centromere divides at early prophase but the chromatids are held together by the segments at the two ends of the centromere. This effect may be due to the stickiness of the heterochromatin blocks in these regions or to some substance secreted by them. After metaphase, these arms separate and the chromosomes move to opposite poles. However, the problem of what holds the chromatids together and allows them to be released at anaphase remains unanswered. The late-replicating nature of heterochromatin may be a contributing factor.

A working hypothesis of the formation of the mitotic spindle suggests that centrioles and asters help in the organisation of the spindle but do not play a direct role in chromosome movement. Centromeric microtubules grow continuously at the kinetochore during prometaphase and metaphase and stop grow-

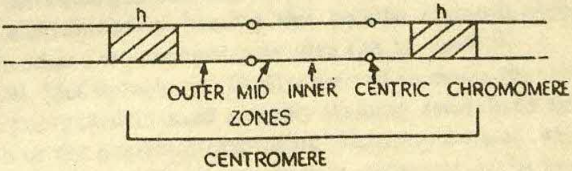

Fig. II : 4.1 Quadruple nature concept

ing during anaphase, carrying with them small particles, acentric fragments, etc. Metaphase plate is formed by pulling the kinetochores towards the opposite poles. Kinetochore fibres are eliminated towards the pole during anaphase, being progressively disorganised towards these regions. The spindle elongation occurs through an increase in length of the continuous microtubules forming them. Cytokinesis, though a separate process, may be triggered by chromosome movements (see Bajer A and Mole-Bajer J 1971 In: *Adv in Cell and Molec Biol* ed EJ DuPraw Academic New York).

CHAPTER II: 5

# SECONDARY CONSTRICTION REGION AND NUCLEOLUS

SECONDARY CONSTRICTION REGION: Non-staining gaps are observed in certain chromosomes in addition to the primary construction regions. These regions are referred to as the secondary constriction regions. Since they are usually the sites for the organisation of the nucleolus, they are also called nucleolus organising regions (N.O.R.) The region of the chromosome distal to this gap, if a small one, is known as the *satellite or trabant*. If a thread is seen to traverse this gap, it is the satellite stalk and the chromosome is a SAT-chromosome. The number of nucleolar organisers in a haploid set of chromosomes varies in different organisms. For example, maize has one and the human cell has five.

RELATIONSHIP WITH NUCLEOLUS: The relationship between the secondary constriction region and the nucleolus has been much-debated. A large number of workers have suggested that these regions are specifically concerned with the formation of the nucleolus. For example,

(i) Heitz (in 1931) propounded that nucleolus is organised in the destained gap between the satellite and the chromosome arm. If the chromosome bearing this specific region is absent, however, other chromosomes may give rise to nucleoli.

(ii) McClintock (in 1934) observed in maize that the nucleolus is organised round a deeply staining specialised body—the knob or the nucleolus-organising element—located at the base of the satellite stalk. In its absence, chromatic blebs are formed all over the chromosomes. She suggested that nucleolar substance is present in all chromosomes and is organised into a nucleolus by the nucleolus-organising element.

(iii) Gates, later supported by Pathak, Dearing and others, observed that the secondary constriction regions form small independent nucleoli at telophase, which later fuse into a single large nucleolus.

On the other hand, Matsuura, Sato, Darlington, De Robertis and others did not accept the relationship between nucleolus and secondary constrictions, and maintained that all chromosomes, if needed, may form the nucleolus.

DIFFERENTIAL STAINING: The evolution of the differential staining methods, including the Feulgen-light green and methyl green-pyronin techniques, which stained the chromosomes magenta and the nucleolus green and *vice versa* respectively, showed definitely the association of the nucleolus with specific areas, the secondary constrictions of particular chromosomes. The maximum number of nucleoli, as observed in telophase of a species, has been found to be constant and to correspond to the number of secondary constrictions present. True diploid species have two identical nucleoli. The number of secondary constrictions and accordingly, the number of nucleoli may be increased by polyploidy and segmental interchanges. A heteromorphic pair of nucleoli indicates heteromorphicity of the chromosomes.

In certain exceptions, sat-chromosomes are relatively rare and the property of organising nucleoli has been ascribed to specific *micro-chromosomes* as in birds.

The secondary constriction region is heterochromatic in nature, though it shows properties different from the other heterochromatic segments of a chromosome. Darlington regarded it to represent a permanent negative phase of allocycly. Morphologically, it differs from the primary constriction in the absence of a centromere and in remaining unstained throughout the divisional cycle. When treated with mercuric nitrate or trichloracetic acid and stained subsequently with Feulgen, the primary constriction region is positively stained while the secondary constriction region, and the euchromatic segments, remain destained.

EVIDENCES FOR ROLE OF N.O.R. IN rRNA SYNTHESIS: The removal of the nucleolus organising region may lead to abnormal behaviour, often resulting in death. This effect is presumably because the organiser is one of the active sites for the formation of a particular RNA, the ribosomal RNA, without which the lifespan of the cell is limited. The evidences in favour of this concept are:

(i) the similarity between the base ratios of nucleolar and ribosomal RNA;

(ii) the ready hybridisation between ribosomal RNA and DNA of the nucleolar region, showing that they are complementary to each other in nucleotide sequence.

(iii) the drastic impairment of protein synthesis in a cell containing a non-nucleolar nucleus, after the supply of ribosomal RNA has been utilised.

(iv) embryonic lethality of mutant forms without a nucleolus organiser in the African clawed toad, *Xenopus*.

GENETIC CONTROL OF NUCLEOLUS FORMATION: The genetic control of nucleolus formation has been demonstrated in hybrids of the plant *Crepis*. Each species of *Crepis* has its own specific nucleolus organiser. When two species are hybridised, the organisers show different synthetic ability in the hybrid environment, one being functional and the other remaining inactive by differential repression of genes. When the latter is transferred to the original conditions, it again becomes active.

DNA CONFIGURATION OF NUCLEOLUS ORGANISER: DNA in the nucleolus organiser region contains many repeated sequences of the DNA (rDNA) that codes for ribosomal RNA and therefore the region has a high potential for DNA amplification, particularly in oocytes. It undergoes many extra replications resulting in a mass of nucleohistone fibres protruding from the nucleolus organiser to form the so-called DNA body. During meiotic prophase, this mass loosens and ribosomal RNA and ribosomal precursors become attached to it. In amphibian oocytes, rDNA of various lengths separate from the nucleolus organiser in pachytene and develop into minute nucleoli at the nuclear periphery. This DNA, after the initial replication, participates in ribosome production. Electron microscopic studies show the active nucleolar chromatin to have a lampbrush-like configuration. Clusters of RNP fibres project from the DNA and are separated by stretches of naked DNA. RNA-DNA hybridisation studies have shown that alternating 18S-28S rDNA sequences are separated by non-transcribed nucleotide sequences—which may correspond to the naked DNA segments.

MORPHOLOGY OF NUCLEOLUS: The nucleolus can be regarded as a detachable product of the chromosome, renewed at each cell cycle. In the course of division, it usually disappears by late prophase, to be reformed during telophase reorganisation. In certain cases, however, it may persist till metaphase or later. Under the light microscope it is a dense body of variable size and shape. The presence of nucleolus within the nucleus was first described by Fontana in 1781. In the living cell, it is a highly refringent body. It is Feulgen-negative after fixation, indicating an absence of DNA, stains with pyronin and absorbs ultraviolet light at 2600Å, due to its RNA-component. It may be surrounded by a ring of Feulgen-positive chromatin, which represents the heterochromatic regions of the nucleolar chromosome. The three main components present in isolated nucleolar fractions are: DNA, RNA and protein.

### ELECTRON MICROSCOPICAL STUDIES OF THE NUCLEOLUS

At the ultrastructural level, four main components may be recognised within the nucleolus:

i) *pars granulosa*, containing mainly RNP granules, 150Å in diameter

ii) *pars fibrosa*, with RNP fibrils, 50Å in diameter

iii) chromatin elements (*pars chromosoma*) and

iv) a heterogenous proteinaceous matrix.

The chromatin elements have been observed in three forms:

i) a nucleolus-associated chromatin, possibly not involved in nucleolus formation, though there are some evidences of its association with certain condensed inactive ribosomal cistrons.

ii) septa-like intranucleolar chromatin, often associated with pars fibrosa, and

iii) isolated or dispersed intranucleolar chromatin threads.

The nucleolar chromatin is a continuous structure and forms a part of the nucleolar chromosome. Nucleoli in normal cells are formed on the same principle as the loops of the lampbrush chromosomes or the functionally active gene loci in interphase chromosomes. A special region of the chromosome, perhaps extending in the lampbrush form or a puff, is responsible for the synthesis of a special type of RNA.

The chromatin responsible for the formation of the nucleolus is its only permanent component. During telophase, the genes located on this segment become active, loops are extended, fibrillar and granular components are synthesised and accumulate in the newly formed nucleolus. During prophase, with the dissolution of the nucleolus, the nucleolar chromatin becomes visible.

The rDNA cistrons, located on the nucleolar chromatin, are mostly redundant and only a few are activated at any particular period. The inactive cistrons are responsible for the condensed appearance of segments of nucleolar chromatin and are late-replicating. The other rDNA cistrons, being in an active and extended state, take part in production of rRNA. The segment where they are located is early replicating. The nucleolus is thus a functional structure and its permanent structural constituent, the nucleolar chromatin, is facultative in nature. The repetitive ribosomal cistrons have a constant location in the chromosomes. The nucleolar heterochromain is thus distinct from both the usual facultative and constitutive heterochromatin, in that it is a segment with fixed location on particular chromosomes and on it are arranged a cluster of repetitious ribosomal cistrons having an identical, definite but *facultative* transcriptional property different from that of the unique genome (see also Ghosh S 1976 *Int Rev Cytol* **40:** 1).

BIOGENESIS OF RIBOSOMES: In considering the nucleolus to have a primary role in ribosome biogenesis, its fibrillar component is regarded to contain the ribosomal RNA genes on which the initial large precursor molecule is synthesised. The RNA molecules contained in the granular part are precursors to the large ribosomal subunits.

In higher organisms, the formation of ribosomal molecules takes place in the nucleolus. A large (45S) RNA molecule is transcribed from the nucleolus organiser, which contains ribosomal DNA (rDNA) and therefore the genes that code for rRNA. This RNA molecule is the precursor of both 28S and 18S ribosomal RNA. Several stages are involved in the formation and maturation of the ribosomal subunits until they are finally completed and reach cytoplasm. Thus the nucleolus plays a key role in the biogenesis of ribosomes.

The stages suggested involve (Fig. II: 5.1):

(i) Synthesis of 45S RNA directed by a set of ribosomal genes located on the nucleolus organiser in alternate sequences of 18S and 28S cistrons interspersed with regions coding for the non-conserved parts of the 45S molecule. Each cistron coding for a 45S RNA molecule contains one each of the DNA segments coding for 28S and 18S rRNA.

(ii) Methylation of the 18S and 28S regions of the 45S molecule occurring during its transcription.

(iii) Combination of 45S RNA with proteins to form a complex sedimenting at 80S. It may contain ribosomal and other accessory types of protein.

(iv) Conversion of 45S RNA to 18S and 32S pieces by cleavage and loss of non-methylated parts.

(v) Incorporation of 5S RNA (transcribed from non-nucleolar genes) into the 65S nucleolar particle.

(vi) Breakage of the 32S RNA to give 28S RNA, with a concomitant change in ribonucleoprotein particle from 65S to 60S.

(vii) Rapid transport of subunits to cytoplasm, their combination with messenger RNA to form polyribosomes and their separation from residual accessory proteins.

Stages in the processing of 45S nucleolar RNA into 28S and 18S ribosomal RNA are possibly:

$$\begin{tikzcd}
45S \arrow[r] & 41S \arrow[r] \arrow[d, dash] & 36S \arrow[r] \arrow[d, dash] & 32S \arrow[r] & 28S \arrow[r] & \text{Cytoplasm} \\
 & 20S \arrow[r] & 18S \arrow[rrr] & & & \text{Cytoplasm}
\end{tikzcd}$$

CONCLUSIONS: With the improvement in methods of electron microscopy and isolation, the nucleoli in eukaryotic cells are seen to vary remarkably in their structure and function. However, their primary purpose is to synthesise ribosomes and possibly polyribosomes as well, though the exact mode of synthesis of mRNA is not yet clear. On the nucleolus organiser, the genes coding for ribosomes number from $10^{2}$ to $10^{4}$ in cells and are separated by spacers. The rDNA genes can be increased signifi-

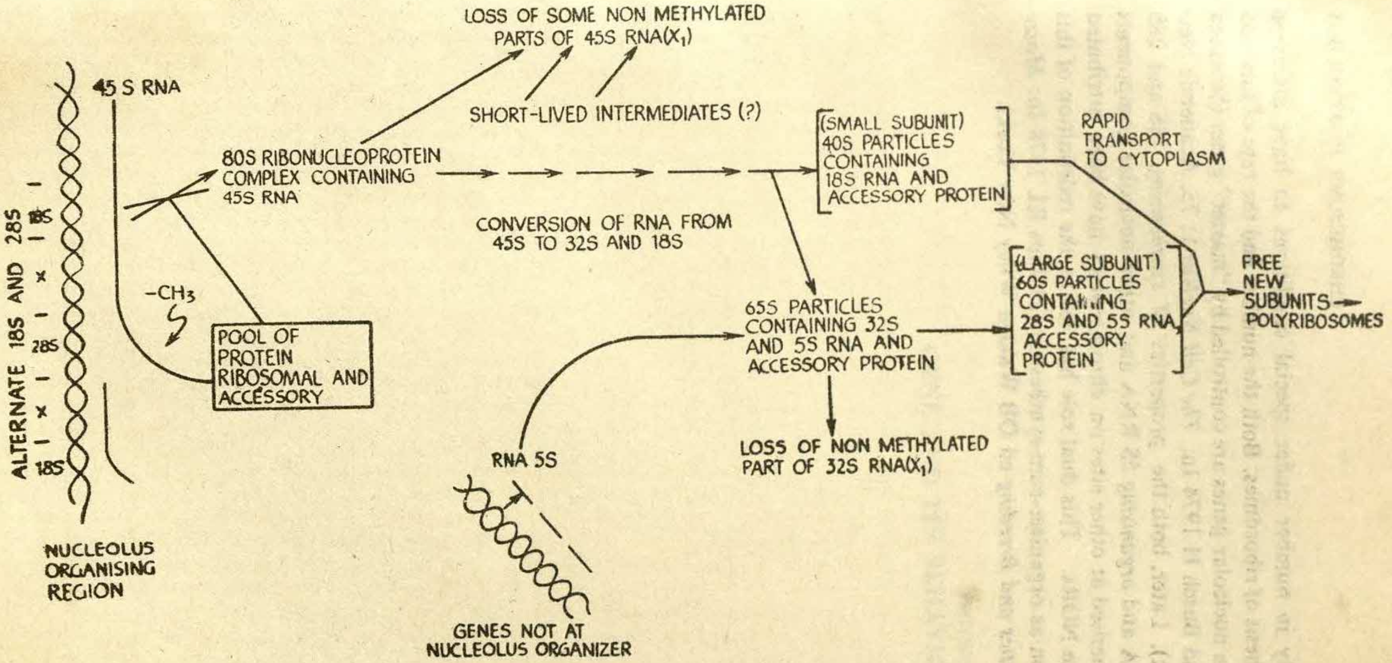
LOSS OF SOME NON METHYLATED PARTS OF 45S RNA($X_1$)
45 S RNA
SHORT-LIVED INTERMEDIATES (?)
80S RIBONUCLEOPROTEIN COMPLEX CONTAINING 45S RNA
(SMALL SUBUNIT) 40S PARTICLES CONTAINING 18S RNA AND ACCESSORY PROTEIN
RAPID TRANSPORT TO CYTOPLASM
CONVERSION OF RNA FROM 45S TO 32S AND 18S
ALTERNATE 18S AND 28S
18S
28S
x
–CH₃
POOL OF PROTEIN RIBOSOMAL AND ACCESSORY
65S PARTICLES CONTAINING 32S AND 5S RNA AND ACCESSORY PROTEIN
(LARGE SUBUNIT) 60S PARTICLES CONTAINING 28S AND 5S RNA ACCESSORY PROTEIN
FREE NEW SUBUNITS → POLYRIBOSOMES
RNA 5S
LOSS OF NON METHYLATED PART OF 32S RNA($X_1$)
NUCLEOLUS ORGANISING REGION
GENES NOT AT NUCLEOLUS ORGANIZER

**Fig. II: 5.1.** **Schematic representation of biosynthesis of ribosomes in eukaryotic cell (after Perry RP 1970 In:** ***Handbook of molecular biology*** **North Holland, (Amsterdam).**

cantly in number under special conditions to have increased synthesis of ribosomes. Both the number and the rate of function of the nucleolar genes are controlled by "master" genes (Smetana K and Busch H 1974 In: *The Cell Nucleus* **1**: 75, Academic New York). Later, both the properties of *synthesising* 18S and 28S rRNA and *organising* 5S RNA and other nucleolar components synthesised at other sites on chromosomes have been attributed to the NORs. This dual role has led to the redefinition of this region as organiser-cum-synthesiser (Phillips RL 1978 In: *Maize Genetics and Breeding* ed DB Walden Wiley New York).

## SECTION III
## GENE AND ITS BEHAVIOUR

tions, to contain under special conditions to [illegible] [illegible] numbers of [illegible]. Both the number and the rate of [illegible] of the nucleolar genes are controlled by [illegible] genes [illegible] [illegible] D 1974 In: The Cell Nucleus [illegible] Academic Press [illegible] York). Later, both the [illegible] of [illegible] 18S and 28S rRNA and [illegible] 5S RNA and other [illegible] [illegible] in other [illegible] chromosomes have been [illegible] [illegible] 1978). This [illegible] has led to the [illegible] of [illegible] region [illegible] (see Phillips R.L. 1978 In: [illegible] [illegible] and [illegible] ed. [illegible] Wiley New York).

CHAPTER III: 1

# INTRODUCTION

## Advancements in methodology

THE advancements in methodology and instrumentation, applied to chromosomes, led to a progressive understanding of the structure and behaviour of *genes*—the ultimate units of heredity—located linearly on the chromosome. In the 1940's and 1950's, the quantitative and qualitative photometric studies, the extraction procedures and enzyme treatments and specific staining of nucleic acids and proteins aided in the study of the major components of the chromosome at metaphase and the chromatin in interphase. With the discovery of the double helix model for DNA, one of the four principal constituents of chromosomes, emphasis shifted towards a more molecular approach to the problems of gene regulation and expression, particularly in prokaryotes. Earlier works on eukaryotes were mainly limited to DNA, due to its relative stability and ease of extraction. The last two decades, however, have seen the evolution of sophisticated techniques for the study of the other ingredients as well, namely RNA, basic and non-basic proteins. These were considerably helped by the availability of large quantities of metaphase cells in pure form, made possible through advances in methods for tissue culture and cell synchronisation. The relevant techniques have been included with the respective chapters (see also Lima-de-Faria A 1983 *Molecular evolution and organisation of chromosome* Elsevier Amsterdam).

## Unravelling of gene concept

The principal landmarks in the unravelling of gene concept, as a sequel to the discoveries initiated by advances in technology, can be grouped into three main phases:

(i) Determination of the chemical composition of the chromosome;

(ii) Identification of the gene substance responsible for heredity; and

(iii) Understanding the pattern of behaviour and regulation of the gene.

### Interdependence of nucleus and cytoplasm

In the functioning of the gene, the interdependence of the nucleus and the cytoplasm is of major importance. The earliest evidence was obtained from the specific inverse relationship between nuclear DNA and cytoplasmic RNA during the divisional cycle observed by Caspersson and later others in the mid-1940s. A disturbance of the DNA-RNA balance resulted in lethality. Further evidences include the formation of the spindle in the cytoplasm under chromosomal control and the effects of radiation exhibited in unirradiated nuclei introduced in enucleated irradiated cytoplasm of bacteria. The final proof is the translation in the cytoplasm of the transcribed genetic code from the nucleus to form proteins.

## CHAPTER III: 2

# CHEMICAL COMPOSITION OF CHROMOSOME

Subsequent to the isolation of nuclear material by Miescher (1874), which he termed "nuclein", now known to be nucleoprotein, nucleic acids and proteins were observed to be normal constituents of all cell nuclei and also of chromosomes.

### A. Nucleic acids

Nucleic acids are contained in all living organisms in the form of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Some viruses, like tobacco mosaic and poliomyelitis virus may contain only RNA in contrast to the bacteriophages and adenoviruses which have only DNA. Both DNA and RNA are present in bacteria and higher organisms. Within the nucleus, DNA occurs in combination with proteins. It has also been recorded in mitochondria, chloroplasts and probably other self-replicating organelles. RNA is located in the nucleolus, nucleoplasm and chromosomes within the nucleus and in the ribosomes within the cytoplasm. Both nucleic acids are long polynucleotides, formed of a series of individual nucleotides linked together. Each nucleotide is further composed of one molecule of phosphoric acid, one molecule of a pentose or deoxypentose sugar and a nitrogenous base (purine or pyrimidine). The combination of a pentose with a base constitutes a nucleoside. The nucleotides are joined by the phosphoric acid, which links the pentoses of two consecutive nucleosides through an ester-phosphate bond. These bonds attach carbon 3′ of one nucleoside to the carbon 5′ in the next one. The remaining free acid group is used to form ionic bonds with the basic proteins, and is responsible for the basophilia of nucleotides, which enables their staining with basic dyes. The pentose sugar is ribose in RNA and deoxyribose in DNA. In the latter, the oxygen on carbon 2′ is missing. The principal pyrimidine bases present are cytosine, thymine and uracil. Thymine is exclusive to DNA and uracil to RNA while cytosine is common to both. 5-methyl cytosine may replace cytosine to a certain extent in some forms of DNA, as in wheat germ. In bacteriophages small quantities of 5-hydroxymethyl cytosine have been found. The purine bases observed in both RNA and DNA are adenine and guanine. In certain types of RNA, like tRNA, methylated forms of the bases like methyladenine or methylcytosine may be observed in large proportions. The alternating double and single bonds between the carbon atoms in the nitrogenous bases can interchange continuously, producing resonance. As a result the bases absorb ultraviolet light at 2600Å. Since adenine pairs only with thymine and guanine with cytosine, their respective ratios are always 1: 1. However the AT/GC ratio in the DNA of different species is variable. AT exceeds GC in higher plants and animals. Variation is much greater in viruses, bacteria and lower plants. Certain regions of a chromosome may be more rich in AT or GC—a property held to account for some of the banding patterns.

DEOXYRIBONUCLEIC ACID (DNA): A *double helix* model was proposed for the spatial *molecular structure of DNA* by Watson JD and Crick FHC (1953 *Nature* **272**:737) following X-ray diffraction studies by Wilkins and Franklin to explain its physicochemical and biological properties. According to this model, two long polynucleotide chains form a DNA molecule. The chains run in opposite directions, forming a double helix around a central axis. The nucleosides are arranged perpendicular to the polynucleotide chains. The latter are held together by mobile hydrogen bonds between the pair of bases facing each other. The sugar moieties in the opposite nucleotides are separated by a constant 11Å. The base pairing between opposing nucleotides is specific due to this distance, one purine base being able to pair with only one pyrimidine and *vice versa.* Adenine may only pair with thymine (AT) and guanine with cytosine (GC), the hydrogen bonds attaching them being two and three respectively. In one polynucleotide chain, different combinations of the bases are present but in its opposing chain within the same helix, the sequence of bases must complement those of the first chain according to the formula:

Phosphoric acid-sugar-adenine=thymine-sugar-phosphoric acid
Phosphoric acid-sugar-cytosine≡guanine-sugar-phosphoric acid
Phosphoric acid-sugar-thymine=adenine-sugar-phosphoric acid
Phosphoric acid-sugar-guanine≡cytosine-sugar-phosphoric acid

Thus the bases of the two strands always complement each other. When a DNA chain replicates, the two strands separate. On each strand, another polynucleotide is synthesised which in its base composition is exactly complementary to the original one serving as a template.

The hydrogen bonds linking a DNA double helix may be broken by high temperature (100°C). The ensuing separation of the strands, associated with an increase in UV absorbancy at 2600Å, is known as *denaturation*. The temperature required for complete denaturation is higher for G≡C than for A=T. Subsequent slow cooling results in *renaturation* or "annealing" of the two complementary strands. This phenomenon has been utilised for the "banding" pattern analysis of chromosomes, through different processes of denaturation and renaturation. The differential composition of chromosome segments, containing different AT/GC ratios, causes differential rates of reannealing. By halting optimally at a particular stage, certain segments, able to renature faster, or resistant to denaturation, exhibit bands (see Section I, methodology). Another application of this property has been *molecular hybridization*, or the formation of hybrid molecules between two different strands of DNA, as for example of mouse and human DNA. The amount of pairing between the two strands, 25% in the above case, indicates the degree of homology between the two strands. Hybrid molecules have also been formed between DNA and RNA strands (Pardue ML and Gall JL 1970 *Science* **268**: 1356).

It can be used to localise specific functional genes on chromosomes. DNA usually exists in the form of a double stranded helix, which may be linear, in the form of a long flexible rod or ribbon, or cyclic with the two ends joined together in a ring. Occasionally it may be single-stranded.

TABLE III : 2.1

Basic biochemical characteristics of all double-stranded DNA

1. DNA consists of two different purines (guanine, adenine) and two different pyrimidines (thymine and cytosine).
2. A nucleotide pair consists of one purine and one pyrimidine [adenine/thymine (AT) or guanine/cytosine (GC)].
3. Nucleotide pairs are connected into a double helix molecule by sugar-phosphate backbone linkages and hydrogen bonding.
4. The AT base pair is held by two hydrogen bonds, while the GC is held by three.
5. The distance between each base pair in a molecule is 3.4 Å, producing 10 nucleotide pairs per turn of the DNA helix.
6. The number of adenine molecules must equal the number of thymine molecules in a DNA molecule. The same relationship exists for guanine and cytosine molecules. However, the ratio of AT to GC base pairs may vary in DNA from species to species.
7. The two strands of the double helix are complementary and antiparallel with respect to the polarity of the two sugar-phosphate backbones, one strand being 3′—5′ and the other being 5′—3′ with respect to the terminal OH group on the ribose sugar.
8. DNA replicates by a semiconservative method in which the two strands separate and each is used as a template for the synthesis of a new complementary strand.
9. The rate of DNA nucleotide polymerisation during replication is approximately 600 nucleotides per second. The helix must unwind to form templates at a rate of 3600 rpm to accommodate this replication rate.
10. The DNA content of cells is variable ($1.8 \times 10^9$ daltons for Escherichia coli to $1.9 \times 10^{11}$ daltons for human cells).

RIBONUCLEIC ACID (RNA) has a structure similar to DNA, except that the sugar moiety is ribose and that uracil replaces thymine. Usually it is single stranded though cases of double-stranded RNA have also been reported.

*Types of RNA*: Three main kinds of RNA have been reported in living cells based on their molecular weight, sedimentation rate on zone centrifugation and function, namely,

(i) Ribosomal RNA (rRNA) is of high molecular weight and is metabolically stable. It is located on the cytoplasmic particles known as ribosomes and forms the bulk of total RNA (about 80%). Based on its molecular weight and corresponding sedimentation rate, it may be divided into two groups. The 50S and 30S ribosomes in *Escherichia coli* yield 23S RNA (mol. wt.

$1.1 \times 10^6$) and 16S RNA (mol. wt. $0.6 \times 10^6$). Their base ratios, base sequences and complementarity with the bacterial genome sites are different. In the mammalian cells, the 30S to 40S and 50S to 60S subunits of the 70 to 80S ribosomes show the presence of two rRNA components. The smaller component has a molecular weight of about $0.7 \times 10^6$ (18S) and the larger one $1.8 \times 10^6$ (28S). Two more RNA's are found in association with the ribosome: 5S RNA having about 120 nucleotides with the larger subunit, from both bacterial and mammalian cells and 7S RNA having about 130 nucleotides with the 28S RNA in the larger subunit from mammalian cells. The origin and role of rRNA in ribosomal synthesis have been given under "nucleolus" in the Section II.

(ii) Transfer RNA (tRNA) or soluble RNA forms about 15% of the total RNA. It has a molecular weight of 23000 to 28000 (4S). It is involved in acting as adaptors for amino acids during protein synthesis. Each amino acid has a corresponding specific tRNA. Both transfer and ribosomal RNA's have been located on mitochondria, distinct from those observed in the cytoplasm.

(iii) The RNA remaining forms about 5% or less of the total amount. It is the messenger RNA (mRNA) and is involved in receiving the message of the genetic code from the DNA for translation during protein synthesis. Its base composition corresponds to the DNA from which it is formed. It is metabolically labile with a molecular weight approaching $0.5 \times 10^6$. It may be identified by its properties of rapid turnover, heterogeneity, complementary base composition with DNA and attachment to ribosomes. It may enter the cytoplasm as a complex with proteins known as *informosomes.*

Certain other types of RNA have been described in cell nuclei and cytoplasm in small quantities, varying in size and stability. In most cases, their function has not yet been ascertained though a few have been found to be precursors of the other functional RNA.

(iv) RNA of a sedimentation rate 6S has been isolated in small quantities from the supernatant fraction after sedimentation of ribosomes in *E. coli.*

(v) Nuclear heterodisperse RNA occurs in nuclei of animal cells together with the precursors of tRNA and rRNA. It has a high molecular weight and may be the precursor of mRNA.

### B. Proteins and polypeptides

Proteins and polypeptides consist of the association in various proportions of some 20 different amino acids attached by peptide bonds (see Table III: 2.2). The different combinations of these amino-acids give rise to a large variety of proteins, determining their biological activity. Essentially an amino acid is derived from an organic acid in which the hydrogen in alpha position is replaced by an amino (—$NH_2$) group, giving rise to amphoteric molecules. Free amino acids in the cell are linked to form poly-

**TABLE III: 2.2.**

**Names of natural aminoacids and their abbreviations***

- Monoamino—monocarboxylic
  - Glycine (Gly)
  - Alanine (Ala)
  - Valine (Val)
  - Leucine (Leu)
  - Isoleucine (Ileu)
- Monoamino—dicarboxylic
  - Glutamic acid (Glu)
  - Aspartic acid (Asp)
- Diamino—monocarboxylic acid
  - Arginine (Arg)
  - Lysine (Lys)
  - Hydroxylysine (Hlys)
- Hydroxyl—containing
  - Threonine (Thr)
  - Serine (Ser)
- Sulphur—containing
  - Cystine (Cys or Cy-S)
  - Methionine (Met)
- Aromatic
  - Phenylalanine (Phe)
  - Tyrosine (Tyr)
- Heterocyclic
  - Tryptophan (Tryp)
  - Proline (Pro)
  - Hydroxyproline (Hpro)
  - Histidine (His)

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*(Taken from Giese AC 1968 *Cell physiology*, 3rd ed, WB Saunder, Philadelphia).

peptide chains by the combination of the acidic group of one amino acid with the basic group of the adjoining one, with the subsequent loss of a molecule of $H_2O$. The linkage—NH—CO— is known as the peptide linkage or bond, the distance between two peptide links being about 3.5Å. The polypeptide chain built of amino acids forms the primary structure of the protein molecule. The nuclear proteins include protamines. histones, acidic proteins and enzymes.

Protamines are simple basic proteins with a very low molecular weight, very rich in arginine. They are found mainly in spermatozoa of some fishes, tightly bound to DNA by salt linkages. Histones are basic proteins with higher molecular weight and greater heterogeneity than protamines. Three main types are observed: high lysine-rich, arginine-rich and slightly lysine-rich. They are bound to DNA by ionic bonds. In higher organisms the DNA : histone ratio in chromatin is almost 1 : 1, an association utilised in the Feulgen-fast green double staining schedule. They have a possible role in the regulation of gene activity, as discussed elsewhere.

Non-histone or acidic proteins are located both in interphase nuclei and chromatin. They are markedly abundant in metabolically active cells. Some of them are linked to DNA. Other remain after the extraction of other proteins by salt solutions and are termed residual proteins. Acidic proteins have a rapid turnover and may possibly play a role in the control of gene expression by reactivating parts of the genomes that are marked by histones. Amongst these, the phosphoproteins are mainly found in the diffuse or active chromatin.

### C. Enzymes

Enzymes are also non-histone proteins. Those involved in gene action are discussed under the respective steps.

### D. Ultrastructure of the nucleus

Under electron microscopy, the interphase nucleus is surrounded by a membrane complex, the so-called nuclear membrane, separating it from the cytoplasm except at the regions of pores. The membrane complex is composed of two membraneous layers separated by a gap (perinuclear cisterna) about 100 to 150Å wide. The nuclear pores interrupt the membranes at intervals. Both membranes are continuous at the margins of the pores. The pores are generally aligned with nucleoplasmic channels situated between the more condensed clumps of chromatin which are attached to the inner membrane. Ribosomes have been found to be attached to the outer surface of the external membrane by the large 60S subunit. The internal membrane, together with the pores and nuclear lamina (a layer of nucleoplasm continuous with the internal membrane), forms a part of the nuclear system. The nuclear pores are often associated with mitotic chromosomes. They are octagonal, 50 to 80 nm in diameter and vary in number from 40 to 140 per square micrometer of nuclear surface in different plants and animals. The pores are occluded by an electron-dense proteinaceous material and enclosed by a circular annulus both on the outside (120 nm diameter) and inside (diameter from 0 to 40 nm). The inner annulus accounts for the selective permeability of the nuclear membrane. Each annulus has eight granules, each 15 nm in diameter, giving it an octagonal shape. Isolated nuclear membrane is seen to be a lipid bilayer with peripheral and integral glycoproteins.

The chromatin attached to the nuclear membrane is usually heterochromatic in nature, some of which may be telomeric. Fine chromatin fibrils of varying thickness are dispersed within the nucleoplasm. Staining for RNA reveals different sizes and types of such fibrils, like interchromatin fibrils, interchromatin granules (25 nm thick); perichromatin granules (50 nm in diameter) associated with the chromatin and other ribonucleo-protein materials present as fibrils. The proteinaceous interchromatin fibrils form the reticulum within the nucleus. The chromatin fibrils exist in a range of thicknesses from 30Å upwards, representing different states of activity.

CHAPTER III: 3

# IDENTIFICATION OF THE GENE SUBSTANCE RESPONSIBLE FOR HEREDITY

## Properties of gene

The nature of the gene component, of the ones listed earlier, responsible for controlling hereditary characters, remained controversial for some time. The gene shows the properties of autosynthesis, heterocatalysis and mutation. The first property leads to a gene replicating in successive cell generations, forming in essence, the basis for transmission of hereditary characters it controls. The second property is the fundamental one involved in gene action, which through a series of chemical reactions, results in the ultimate expression of the characters within the organism. Mutation is a change in the nature of the gene. It is finally reflected as an alteration in its effect and forms the source of variations besides recombination for evolution. In addition, the gene substance must exhibit a very wide diversity in form to account for the innumerable diversities in the characters of organisms occurring in nature. Since each character is the ultimate expression of a chain of reactions initiated at the gene level, obviously each gene must be a chemically unique entity.

ARGUMENTS IN SUPPORT OF PROTEIN: Of the two most likely candidates for the position of gene substance—nucleic acid and protein—protein received considerable support from earlier workers. Nucleic acid was regarded as unsuitable due to its comparatively uniform chemical structure, being made up of sugars, phosphates and bases and due to the observation that it appeared only during certain stages of the divisional cycle and was therefore not a permanent entity. On the other hand, protein showed a highly complex structure, formed of polypeptide chains, which in turn, consisted of a number of amino acids. The wide range of diversities possible in the protein molecule, due to different combinations of amino acids, appeared to fulfil the diversity required in the gene substance.

ARGUMENTS IN SUPPORT OF NUCLEIC ACID: Refinements in methodology, as discussed earlier, demonstrated that the nucleic acid, far from being transitory, forms an essential part of chromosome and is present in all stages of the cell cycle, including the interphase or so-called "resting" nucleus. The wide variety of combinations of base pairs in the nucleotide sequences refutes unequivocally the limitation of "monotony" attributed to its structure.

EVIDENCE IN SUPPORT OF DNA: The evidences which have shown beyond doubt that, of the chromosomal components, DNA is the gene subtance are based on a series of discoveries involving bacterial transformation, transduction and viral multiplication. Along with increase in gene dosage following polyploidy, there is a direct increase in DNA content. In addition, enzyme digestion and ultraviolet absorption methods have indicated the responsibility of DNA in maintaining the structural integrity of the chromosome.

(*a*) *Bacterial transformation:* Pneumococci can be grouped into different types on their ability to synthesise specific polysaccharides. Under particular conditions, a specific strain of pneumococci lost their property of synthesising their corresponding polysaccharide. As a result, this colony appeared "rough" in contrast to other "glistening" colonies formed *in vitro* by the other strains which bore their gummy polysaccharide capsules. A living culture of such "rough" strain was injected into mice with a dead culture of the capsulated strain. On examination of the organisms subsequently recovered from the mice, they were found to be living virulent pneumococci with capsules. Evidently therefore, the non-capsulated pneumococci have acquired the property of synthesising polysaccharides to form capsules from some material in the dead strain of capsulated pneumococci. Later, the same pneumococcal transformation was carried out *in vitro* by adding a cell-free extract of the killed capsulated strain to a culture of the non-capsulated one. Such transformed pneumococci did not revert to the original type (Fig. III: 3.1). The chemical nature of this extract was shown by Avery, McLeod and McCarty (1944) to be DNA alone. DNA extracted from the capsulated strain could transform the uncapsulated "rough" strain to the capsulated one which maintained the property through generations and produced identical DNA. It therefore showed the properties of reduplication and of induction of the inheritable capacity of capsule synthesis—two characteristics attributed commonly to the gene. The phenomenon of transformation was later observed in a wide range of bacteria. Other characters than capsule formation could also be transferred, including drug resistance to various antibiotics.

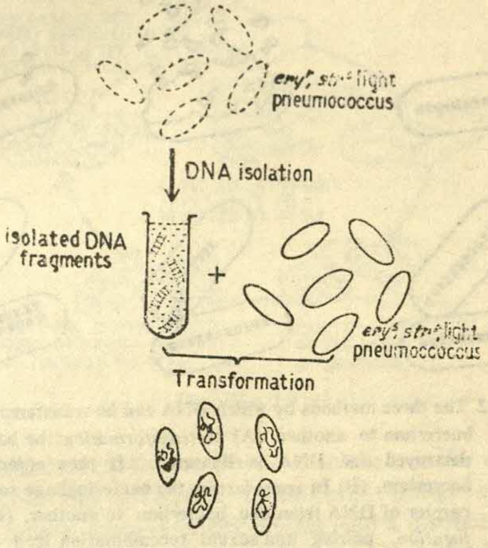


Fig. III : 3.1.

(*b*) *Viral multiplication*: Further evidence of the DNA nature of the gene was provided by a study of viral multiplication. Bacteria are attacked by specific types of viruses called bacteriophages. A bacteriophage has a very peculiar shape. It resembles an injection syringe, with a long hollow body and a

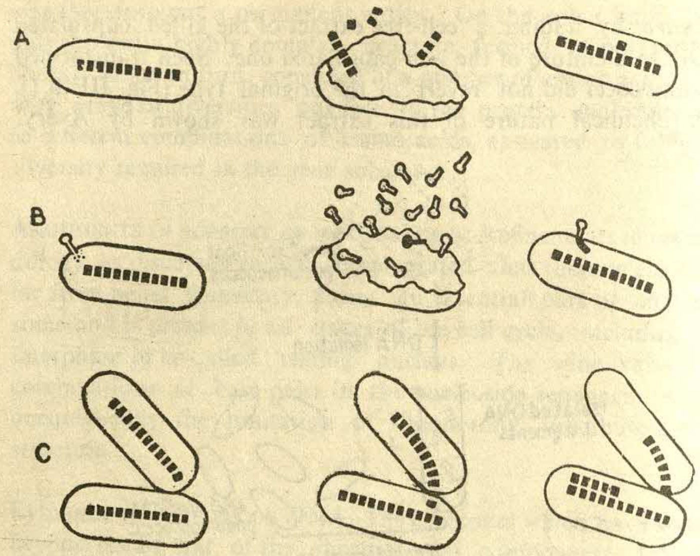


Fig. III : 3.2 The three methods by which DNA can be transferred from one bacterium to another. (A) In *transformation* the bacteria are destroyed and DNA is liberated. It then enters the next bacterium. (B) In *transduction* the bacteriophage serves as the carrier of DNA from one bacterium to another. (C) In *conjugation*, pairing and sexual recombination lead to a direct transfer of DNA (from De Robertis *et al.* 1970).

pointed hollow tail. The whole body or the syringe is made of protein and the comparable fluid inside this body consists of DNA. The various types of phages are distinguished by the composition of their protein coats. When a bacterium is infected, the phage attaches its pointed end to the outer wall of the bacterium, the wall between the phage and the bacterium dissolves and the body fluid of the phage containing DNA alone is injected into the body of the bacterium, which has been confirmed through $P^{32}$ and $S^{35}$ labelling as well. After some time numerous new phages are formed through multiplication inside the bacterium. They come out through lysis of the bacterium resulting in the death of the latter. Thus the new phages—their DNA fluid and protein bodycoat—are all formed out of the substance injected into the bacterium which contains only DNA

Hence DNA may be regarded as the substance responsible for hereditary transmission (Fig. III:3.2 and 3).

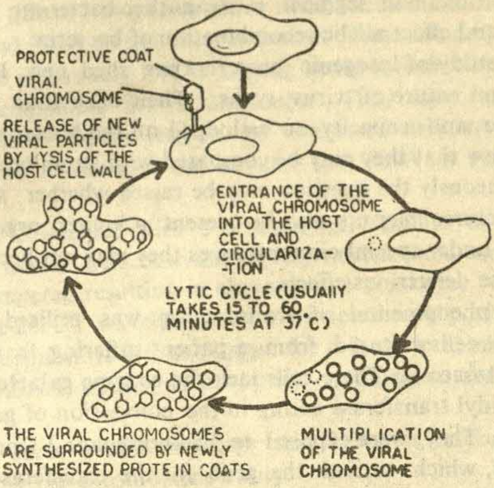

PROTECTIVE COAT
VIRAL CHROMOSOME
RELEASE OF NEW VIRAL PARTICLES BY LYSIS OF THE HOST CELL WALL
ENTRANCE OF THE VIRAL CHROMOSOME INTO THE HOST CELL AND CIRCULARIZA-TION
LYTIC CYCLE (USUALLY TAKES 15 TO 60 MINUTES AT 37°C)
THE VIRAL CHROMOSOMES ARE SURROUNDED BY NEWLY SYNTHESIZED PROTEIN COATS
MULTIPLICATION OF THE VIRAL CHROMOSOME

Fig. III : 3.3

(*c*) *Transduction* is the term applied to the transfer of genetic material from one bacterium to another through a bacteriophage. In certain cases infecting phages lead a distinct existence within the host bacterium. They may be present in the bacterium for a long time without hurting it. In such cases, the phage nucleic acid is found to be associated with the bacterial chromosome at a definite position along it—a phase known as the *prophage*. The prophage divides at the same time as the bacterial chromosome and maintains a similar sequence. As the nucleic acid thread of the prophage is short as compared to the bacterial chromosome, it looks and behaves like a gene. This behaviour of the prophages provides further proof that DNA is the gene substance.

Under certain constant external conditions the prophage is harmless and bacteria reproduce normally. If, however, the environmental conditions change, the passive prophage takes command and forces the bacterium to undergo complete lysis. Bacteria that carry such prophages are known as lysogenic bacteria and phages that can behave as prophages are known as *temperate phages*. Such temperate phages while forming new phages take a part of the DNA of the host chromosome and transmit this small segment into another bacterium by further attack, and effect allelic recombination of bacteria.

The study of lysogenic bacteria has shed new light on the origin and nature of virus types. Their behaviour resembling the gene and capacity to be lodged on the bacterial chromosome show that they may be compared with an unlocalised gene. Simultaneously the question may be raised whether similar hidden structures may not also be present in higher organisms and whether under special circumstances they may not become active and cause deleterious effects.

The phenomenon of transduction was utilised in human fibroblast cells cultured from a patient suffering from congenital galactosaemia. These cells lack the enzyme galactose 1-phosphate uridyl transferase acting in the conversion of galactose to glucose. They were exposed to bacteriophage λ from *Escherichia coli*, which carried the gene for the transferase. Subsequently, the fibroblast cells were found to be able to synthesise the missing enzyme. Obviously the bacteriophage λ had transferred the piece of DNA responsible for the enzyme from the DNA thread of *E. coli* and incorporated it into the genome of the human fibroblast. Labelling the RNA of the recipient cells showed that 0.2% of them could hybridise with λ DNA indicating that the amount transferred to them came from the λ thread. This piece of incorporated DNA thread formed an integral part of the human chromosome since the ability to synthesise transferase was inherited in the succeeding cell generations.

Transduction proves that (i) DNA is the gene substance and (ii) the DNA's of phages, bacteria and human beings are essentially the same and can be incorporated into each other. A future application of this method may be in gene therapy in transferring a requisite gene into the genomic thread of a deficient cell strain (see Sinsheimer RL 1970 *Impact of science on society* **20**: 279).

DNA AND STRUCTURAL INTEGRITY OF CHROMOSOMES: Certain evidences were also obtained to show that DNA is responsible for maintaining the structural integrity of chromosomes; namely,

(i) On treating lampbrush chromosome with deoxyribonuclease, the entire chromosome structure could be disorganised. The greatly extended thread-like loops of this type of chromosomes consist of two parallel strands 100-150Å in diameter. Their structure is not affected by proteases, or ribonucleases indicating that proteins and RNA do not enter into their formation and that the threads are exclusively DNA. During active metabolism, certain particles accumulate around the loops. They later move into the cytoplasm and are seen to be composed of RNA. It provides another evidence of the concept that RNA is synthesised by DNA.

(ii) By treatment with 5-bromouracil (BuDR or FuDR), which are nucleic acid base analogues, nucleic acid metabolism can be hampered resulting in chromosome breakage.

(iii) At 2650Å wavelength of ultraviolet rays purine shows heavy absorption. If it is prolonged for a long time, nucleic acid molecule (DNA) undergoes disruption resulting in chromosome breakage.

These three evidences show that DNA is responsible for maintaining the chromosome entity.

In certain viruses, however, like the tobacco mosaic virus in plants, the genetic substance is RNA.

CHAPTER III : 4

# PATTERN OF BEHAVIOUR AND REGULATION OF THE GENE

Having identified DNA as the gene substance from the evidences cited previously, the next stage is to study its mode of action during autosynthesis, heterocatalysis and mutation—the three principal functions ascribed to the gene.

## A. Replication

Replication of a DNA molecule gives rise to two identical daughter molecules, fulfilling the criterion of autosynthesis. Each cell produced as a result of mitotic division receives exactly the same complement of DNA—and, therefore, the same set of genes—both quantitatively and qualitatively, as was contained in the parent. This amount is constant in the resting somatic (2n) nuclei of any one species, double in the cells about to divide and half in the germ cells containing *n* number of chromosomes. Measurements of DNA content during the divisional cycle shows that DNA synthesis or replication takes place only at a specific portion of interphase, the so-called S-phase, which is preceded and followed by two periods, $G_1$ and $G_2$ respectively, when there is no DNA synthesis.

MECHANISM: During replication, the double helix of DNA unwinds into two polynucleotide strands. Each strand then acts as a template on which a fresh complementary strand is synthesised, matching the complementary base pairs on each nucleotide. The nucleotides are then linked together into a polynucleotide by an enzyme known as DNA-dependent DNA polymerase. It was purified from *E. coli* (see Kornberg A 1968 *Scient Amer*

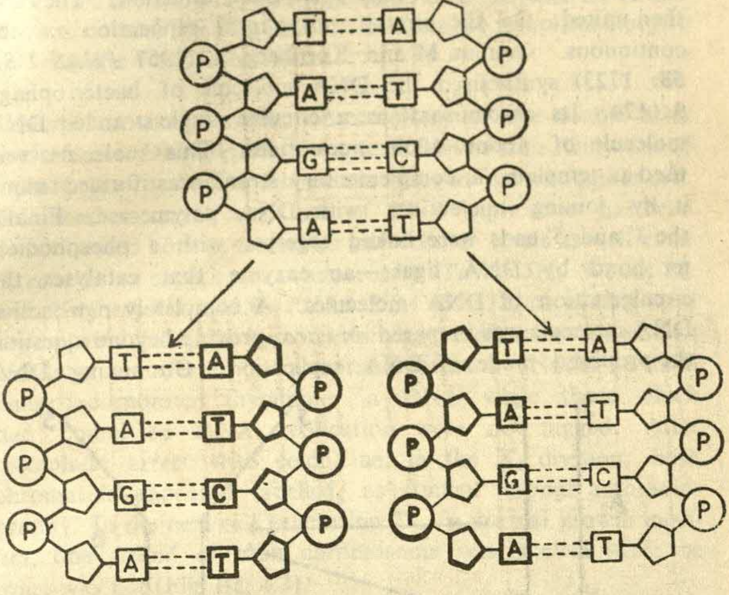


Fig. III : 4.1 The mechanism of DNA replication. The two parent molecules in the top separate by the opening of the hydrogen bonds. In the figure below, the two new strands that have been synthesized, with base compositions complementary to the parent DNA strands are indicated by bold outlines (after Kornberg A, 1962, in Ciba lecture in microbial biochemistry, John Wiley and Sons, New York).

**224:** 69 for review). It is a protein of mol. wt. 109000 in the form of a single polypeptide chain. As the hydrogen bonds linking the two strands of a DNA double helix are weak, they do not need any enzyme mechanism for separation. The unwinding of the strands at the region of replication and the rewinding of the daughter strands after replication take place simultaneously (Lewinthal C and Crane HR 1956 *PNAS USA* **42**: 436). The strands at one end start unwinding and their complementary strands are synthesised at the same time, the new helices forming a Y-shaped fork with the as-yet-unwound portion of the parent helix. The DNA polymerase is seen to polymerise the nucleotides in only one direction, from the 5′ end to the 3′ end. When the two strands unwind, the 3′ end of one and 5′ of the other are opposed. Short pieces of DNA are synthesised on the strand ending in 5′ from the 5′ to the 3′ direction. They are then united. On the strand ending in 3′, replication may be continuous. Goulian M and Kornberg A (1957 *PNAS USA* **58**: 1723) synthesised the DNA molecule of bacteriophage 9×174. Its chromosome is a circular single-stranded DNA molecule of about 6000 nucleotides. This molecule was used as template, a complementary strand was formed along it by joining nucleotides with DNA polymerase. Finally the 3′ and 5′ ends were linked together with a phosphodiester bond by DNA ligase—an enzyme that catalyses the circularisation of DNA molecules. A completely new active DNA molecule was prepared *in vitro*, proving beyond question the proposed mode of DNA replication. On mixing DNA

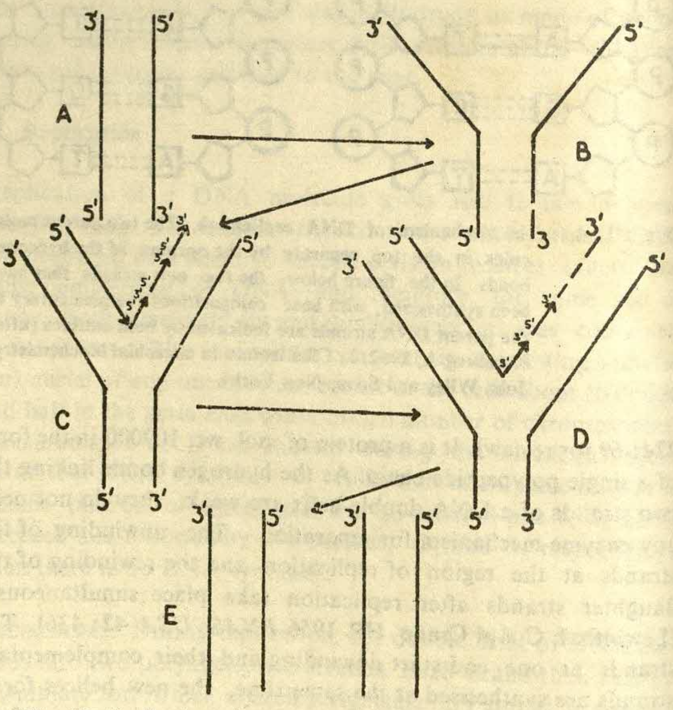


**Fig. III : 4.2 Replication of DNA using DNA polymerase. The chain ending in 5′ is replicated continuously, while the one ending 3′ is formed in a series of fragments which are then joined together.**

polymerase with the triphosphates of the four bases (dATP, dGTP, dCTP and TTP) in the presence of a template DNA and a few co-factors like Mg ions, a DNA strand complementary to the template was synthesised.

SEMICONSERVATIVE NATURE: A corollary of the double helix model is that DNA replication is semi-conservative. Only one of strands, that is, half of a molecule goes to each daughter cell. The semi-conservative nature of DNA replication has been demonstrated both at molecular and cellular levels as well as in *E. coli*. In an experiment conducted by Taylor JH, Woods PL and Hughes WL (1957 *PNAS USA* **43**: 122) plant cells were first grown in a medium containing radioactive thymidine and later transferred to a normal medium. Nuclei in the S phase incorporated thymidine in DNA while those which had completed DNA replication were not tagged. After metaphase arrest with colchicine, in the $X_1$ division, both chromatids appeared labelled, as studied through autoradiography. In the next cell generation $X_2$, in normal growth medium, one strand of each chromosome was labelled while the other was not (Fig. III: 4.3).

It was concluded that prior to replication each chromosome behaves as if it has two units of DNA along its length. During S phase, two new labelled units are built along the original DNA threads. Thus each chromatid includes an original non-labelled strand and a new labelled strand complementary to it. In the second S phase, since the medium contains no isotopes, the labelled and unlabelled strands separate into labelled and non-labelled sister chromatids. It was confirmed by Messelson and Stahl through $N^{15}$ labelling and caesium chloride density gradient centrifugation. These observations, known to be valid for all chromosomes, also support the proposal that the chromosome is uninemic, each chromatid containing only one double helix of DNA (see Callan HG 1973 *Br Med Bull* **29**: 192; Davidson JN 1972 *The biochemistry of nucleic acids* 7th ed Chapman and Hall London, for reviews).

LATE REPLICATING DNA: In cytological terms, interphase exists in three forms: (i) the euchromatic state where DNA is decondensed in interphase, active in transcription and replicates early in the S phase, (ii) the facultative heterochromatic form, where DNA may be condensed, inactive in transcription and late replicating or the same DNA may be present in the euchromatic state, (iii) the obligatory or constitutive heterochromatin, where it always remains condensed, inactive in transcription and late or early replicating.

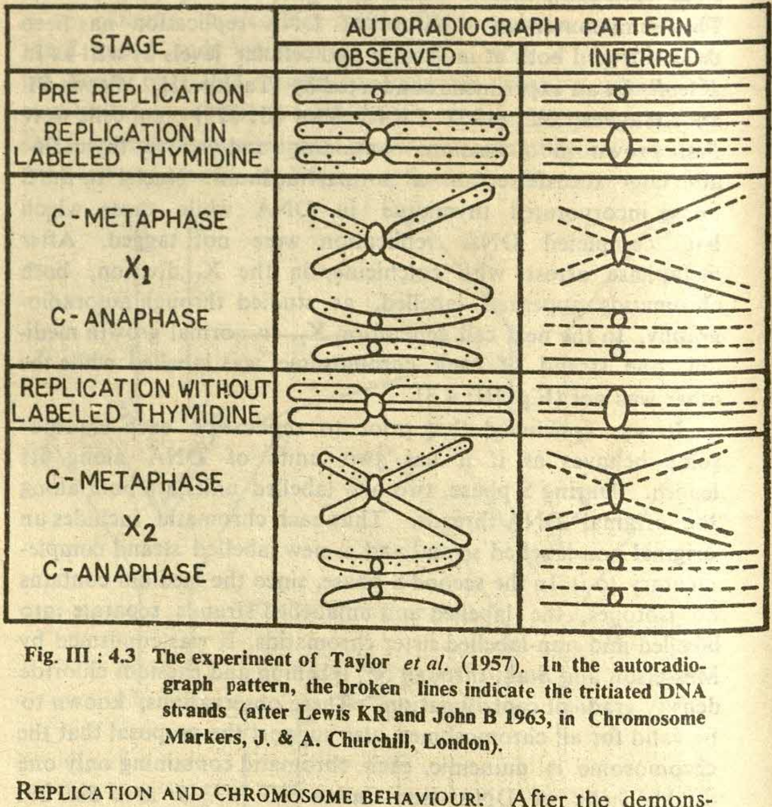


**Fig. III : 4.3** The experiment of Taylor *et al.* (1957). In the autoradiograph pattern, the broken lines indicate the tritiated DNA strands (after Lewis KR and John B 1963, in Chromosome Markers, J. & A. Churchill, London).

**REPLICATION AND CHROMOSOME BEHAVIOUR:** After the demonstration of the semiconservative nature of replication of DNA thread, the nature of chromosome and DNA was extensively studied. The chromosome before replication behaves as a two-stranded chromatid analogous to a double helix with each new strand being synthesized on the outside of the old strand. DNA in a metaphase chromosome is regarded to exist as a double helix which replicates in separate segments along this strand. Replication proceeds at the same rate in both directions from an initiation point in each segment or replicon (see Cairns J 1966 *Scient Am* **214**: 37).

INITIATION OF REPLICATION: The initiation of DNA synthesis from the $G_1$ phase, following a stimulus or removal of inhibition, requires many hours. Several authors suggest that initiation is dependent upon a particular cell mass. Others have attributed it to thymidine kinase activity which rises near the beginning of DNA synthesis. In any case, thymidine kinase is not on the main pathway and there are several cell types which do not show this enzyme, but are capable of DNA synthesis. The appearance of enzymes necessary to support DNA synthesis is coordinated with the initiation of synthesis, but it is unlikely that any of these enzymes is involved in the regulation of initiation.

In prokaryotes, the initiator protein is responsible for regulation of DNA synthesis, which may be applied to eukaryotes as well. According to this hypothesis, the initiation of replication depends on the synthesis of a particular RNA message and the corresponding protein. Presumably, the initiator protein accumulates during cell growth and when present in sufficient amount, it interacts with DNA at the replicator region of each replicon, to make replication permissible. In eukaryotes, the evidence for the presence of initiator protein is of a general nature. The importance of protein synthesis for switchover from $G_1$ to S phase has been visualised. Inhibition of DNA synthesis occurs if RNA synthesis is inhibited in $G_1$ by antibiotic treatment. Similarly, even after DNA synthesis has been initiated, the inhibition of RNA synthesis immediately results in the rapid depression of DNA synthesis. For late initiating segments, initiator proteins may be visualised to be formed at a later phase. The exact nature of protein responsible for initiating replication is not yet fully established. Evidences so far obtained indicate that protein synthesis is essential for DNA synthesis to be initiated.

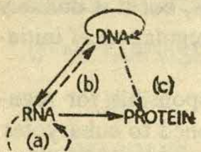
## B. Transcription and translation

As a carrier of genetic information, the second function to be fulfilled by DNA is that of *heterocatalysis*, that is, to initiate a chain of reactions so that the information coded on it is converted into proteins, resulting in expression of the character controlled. The genetic information is transferred in two stages:

(i) *Transcription* of this information into different RNA molecules; and

(ii) *Translation* of this information transcribed in RNA into the different protein components, including the enzymes, of a cell. The two steps may be summarised as,

$$\text{Replication}\rightleftharpoons\text{DNA}\xrightarrow{\text{Transcription}}\text{RNA}\xrightarrow{\text{Translation}}\text{Protein}$$



*Central dogma*: It has been further modified by Crick (FHC 1970 *Nature* **227**: 661) as shown in the diagram. The solid arrows in the diagram indicate general transfers; dotted ones refer to special cases like: (a) synthesis of viral RNA on viral RNA template; (b) action of RNA-independent DNA polymerase (see Temin HN and Mizutuni S 1970 *Nature* **226**: 1211) and (c) an as yet unexplained reaction.

*Variations*: Variations from the central dogma occur when:

(i) RNA is synthesised from an RNA template in virus-infected cells;

(ii) DNA is synthesised from an RNA template in cells infected with turnover viruses; and

(iii) DNA acts directly as the template for protein synthesis without mRNA as intermediary in the presence of an antibiotic, neomycin.

In the ultimate analysis all characters of structure and behaviour are controlled by genes. The expression of a character is the culmination of a series of chemical reactions initiated at the gene level resulting in the formation of a particular protein designed for the purpose. Since each step in a chemical reaction is known to be catalysed by an enzyme, obviously therefore, the synthesis of each enzyme must also be regulated by a specific gene.

*Problems*: The two major problems facing geneticists, after the identification of DNA as the gene substance, were:

(i) to confirm the postulate that each chemical reaction and therefore, each enzyme, is controlled by a gene, or a specific portion of the DNA thread in the chromosome; and

(ii) to find out the way through which the gene, located on a chromosome inside a nucleus, is able to transmit its orders to the cytoplasm where protein synthesis takes place.

GENE-ENZYME RELATIONSHIP: Knowledge of gene-enzyme relationship stems largely from research with a number of different micro-organisms. Many concepts of this relationship were derived from experiments with *Neurospora* initiated by Beadle GW and Tatum L (1941 *PNAS USA* **27** : 499).

*One gene–one enzyme*: The evidences for one gene–one enzyme hypothesis include the following:

A wild strain of *Neurospora* can synthesise all the amino acids essential for the formation of its protein. Normally, this mould can grow in a 'minimal medium' containing sucrose, nitrate, minerals and only one vitamin—biotin. However, if spores of wild strain of *Neurospora* are treated with a mutagen, some of the spores will be unable to grow on the minimal medium and will require the addition of some other substance, such as amino acid citrulline or vitamin niacin. In a living system the synthesis of organic molecules, such as amino acids, is termed biosynthesis. It entails in most cases a series of stepwise biochemical reactions, each catalysed by an enzyme. For example, the cycle from ornithine to protein can be summarised as follows:

$$\begin{tikzcd}
\text{precursors of ornithine} \arrow[r] & \text{ornithine} \arrow[r, "+CO_2"] & \text{ornithine carbonate} \arrow[r, "+NH_3"] & \text{citrulline} \arrow[d, "+NH_3"] \\
 & & \text{protein} & \text{arginine} \arrow[l]
\end{tikzcd}$$

There are several mutant strains of *Neurospora* that are unable to synthesise arginine. Since there are different steps in the synthesis of arginine, a mutation at any one of them results in a block in arginine biosynthesis. When each mutant type is crossed with a normal wild type, the latter progeny shows inheritance indicating a single gene difference. One class of mutant was found to grow only when supplied with arginine, another either on arginine or on one of its precursors—citrulline, a third not only on arginine and citrulline but also on ornithine. An arginine-requiring mutant that can grow in the presence of ornithine is able to carry out the reactions from ornithine through citrulline to arginine. Therefore a mutant of this type will also grow when given either citrulline or arginine. Obviously gene mutation in this class of mutants results in a block in one of the reactions that precedes the synthesis of ornithine. Similarly, a mutant that can grow in the presence of citrulline or arginine, but not ornithine, must be unable to convert ornithine into citrulline, and accordingly a mutant that can utilise only arginine must possess a block between citrulline and arginine. The evidence derived from examining the blocks in arginine biosynthesis shows that each of the three mutant classes has lost one of the three different biochemical reactions. A different enzyme is required for each of the steps in the biosynthesis of arginine. Therefore the functional distinction between wild and mutant types resides either in the loss of ability to produce one of these three essential enzymes or in the production of an altered enzyme. Thus in this case, the relation between gene and enzyme is 1 : 1.

One of the earliest investigations of biochemical mutations concerned the metabolic disease of man known as alkaptonuria. This metabolic error is inherited as a recessive trait. The affected individuals, in addition to having bone disabilities, are unable to metabolise a substance called homogentisic acid into acetoacetic acid, which in turn is excreted in the urine. It oxidises upon exposure to air causing the urine to become black. The chemical reaction by which homogentisic acid is converted to acetoacetic acid requires the presence of an enzyme that is either inactive or absent in alkaptonurics. Pedigree data, where available, show the inheritance as a monogenic character. A series of similar cases of inherited disorders, due to malfunctioning or absence of a gene along a metabolic pathway, has been recorded in man. They are known as inborn errors of metabolism and have been discussed separately (Chapter IV: 5).

*One gene–one polypeptide*: The earlier workers, cited above, showed that each amino acid synthesis is controlled by a single gene and the relationship between gene and enzyme is 1 : 1. The data implied that when applied to higher organisms the concept of a gene controlling a single character must involve units which are separable. Each character is the expression of several reactions and in its constituents involves a number of proteins. The latter in turn are made up of a number of amino acids. Evidently if a gene is responsible for the synthesis of a single amino acid then this is not the unit which was visualised by Mendel.

*Concept of gene-cistron, muton, recon*: The ultimate unit concept of gene of Mendel suffered a serious setback following the work of Benzer (S 1962 *Scien Amer* **206** : 70) on lower organisms. Several triplets of base pairs in the DNA which were necessary for the synthesis of several amino acids constituting a polypeptide chain were observed to be located adjacent to each other in a definite sequence, in a functional unit (cistron) within a chromosome. Therefore the *cistron*, which is responsible for a polypeptide is comparable to the *gene of Mendel*. But the cistron is complex and is composed of a large number of triplets (at least 200 triplets in a bacteriophage). Thus if gene is compared with cistron, the former can be visualised as a chemically long molecule, the ultimate unit of which is a single base capable of undergoing mutation, and so termed by Benzer as *muton*. The length of a muton, which is a single base, is fixed for all organisms. The length of cistron on the other hand varies within a chromosome depending on the number of triplets needed for the production of their corresponding polypeptides. Similarly the length of *recon*, i.e., unit of recombination, also undergoes considerable variation. All these evidences distinctly suggest that the gene as visualised by Mendel is certainly not the ultimate unit of inheritance (see also Beermann W, Reinert J and Ursprung H 1972 ed *Results and Problems of Cellular differentiation* **4** : Springer Berlin).

Of the two theories regarding gene-enzyme relationship, that of enzymes being directly produced by genes has been discarded. The byproduct theory, too is not absolutely correct as it suggests that chromosomes while dividing throw off certain byproducts to react with cytoplasmic precursors to give specific enzymes. Genes, through specific chemical reactions, produce certain products which certainly act as intermediaries in enzyme synthesis.

**GENE IN DIRECTING THE PRODUCTION OF ENZYMES:** The genetic material is located in the nucleus, but the bulk of protein synthesis occurs not in the nucleus but in the cytoplasm. Amino acids labelled with radioactive tracers may be incorporated into protein without passing into the nucleus. Cells may even continue to manufacture proteins for a period of time through already available transcribed messages after the nucleus has been removed. In cell-free systems, protein synthesis can often be demonstrated even though it occurs at a much slower rate than in intact cells and that also for a short period, when even DNA is absent. Such evidences demonstrate that DNA does not regularly participate directly in the fabrication of proteins. Rather, the information encoded in the genetic material must first be transferred to an intermediary which is less localised geographically.

*"Messenger" RNA*: All evidences indicate this intermediary to be a special kind of RNA. Although RNA is found throughout the cell, little if any of it is synthesised in the cytoplasm. RNA precursors labelled with isotopes appear first in nuclear RNA and only later in cytoplasmic RNA. Moreover, all cell-free systems for protein synthesis contain RNA and are inactivated when they are hydrolysed with the enzyme ribonuclease, which digests RNA. RNA is, therefore, the likely candidate for the role of 'messenger'. If so, the transfer of information from DNA to protein involves two steps, DNA to RNA, and RNA to protein.

*Transcription*: The transcription of DNA information to RNA appears

$$\text{DNA}\xrightarrow{\text{transcription}}\text{RNA}\xrightarrow{\text{translation}}\text{protein}$$

not to constitute a major problem. An RNA strand could be manufactured by a DNA strand in much the same way that a complementary DNA strand is formed by hooking together ribonucleotides that have been added in the form of triphosphates. A cytosine base in DNA would be faced by guanine and a thymine base in DNA by adenine and the entire sequence linked with the help of an enzyme *RNA polymerase*. The major difference between autosynthesis (forming more DNA) and heterocatalysis (forming RNA) would be that the complementary strand would be made up of ribonucleotides rather than deoxyribonucleotides and that uridine rather than thymidine would be opposed to adenine. The immediate factor determining whether the DNA is to be copied or transcribed will depend on the enzyme involved, DNA polymerase and RNA polymerase respectively, both dependent on the presence of DNA for activity.

*DNA-RNA hybridisation*: RNA originating from DNA would be expected to have a specific purine and pyrimidine base sequence. It should pair after its formation, base for base, with single strands of its parental DNA. This molecular 'hybridization' can occur through a technique in which DNA-RNA mixture is heated (denaturation or formation of single strands) and then gradually cooled (renaturation or formation of double strands). If the base sequences in this mixture are complementary, close fitting double strands —'hybrids' are formed which protect the RNA from the degenerative action of the enzyme ribonuclease.

So far, all RNA fractions, messenger, ribosomal and transfer, have been shown to successfully hybridise with DNA of the same species indicating their origin from the master template of DNA. On the other hand, since many hereditary differences exist between species, interspecific hybrid DNA-RNA combinations would not be expected, and this is generally found to be the case.

STRUCTURAL CONTINUITY OF EUKARYOTIC CHROMOSOMES: Whether the eukaryotic chromosome represents a single long DNA molecule or several small DNA molecules, arranged end to end as several replicons, has been a controversial issue. Serial arrangement of DNA molecules has also involved the assumption that linker molecules are present in between the different replicons.

*Linkers* : Theories have been proposed suggesting the presence of (a) protein, (b) RNA, and (c) the divalent cations $Ca^{++}$ and $Mg^{++}$, serving as linkers. In order to test these hypotheses, enzyme digestion tests were applied, and it was observed neither proteases nor ribonuclease digestion can disrupt the chromosome structure. Similarly, a removal of cations through chelation has also resulted in maintaining the structural integrity of the chromosomes. These evidences show that the replicons in the DNA molecule are not attached to each other by means of any such linkers. Several authors have observed that DNA molecules, $93\mu$ long in sea-urchin sperm and $500\mu$ long in HeLa cells, can remain intact without showing any evidence of linkers. No doubt, serine molecules were observed to be present in purified lymphocyte DNA, but no evidence has later been found to indicate their presence in the DNA backbone. In fact, the concept of the presence of non-DNA linkers is now mostly abandoned. Linkers between replicons may exist just as linkers exist between two operons, i.e., through nonsense triplets or spacers as in RNA cistrons (see also sect II : chapter 3).

*Multireplicon model*: However, even though the linker concept is invalidated, the presence of replicons in a chromosome has been clearly demonstrated in several organisms. The best evidence comes from Chinese hamster chromosomes where, following 10 minutes labelling, several sectors show label even in a single arm at metaphase. Such simultaneous replication in several segments of a chromosome is only possible if there are independent replicons. In other words, there may be multiple initiation sites. The number of replicons in a chromosome may vary. In *Drosophila* it has been estimated that in four chromosomes more than 200 replicons are present. The independent extrachromosomal genetic replicating units may be present in prokaryotic systems within episomes and plasmids.

The advantages of the replicon concept are manifold. It provides a suitable model for independent replication of different chromosome segments. Thus, early and late replication can be easily accounted for. Similarly, the termination and initiation points of replicons may suggest loci for genetic crossing-over. Translocation of segments, even reciprocal and at an intercalary level, can be explained through such a mechanism.

CHAPTER III: 5

# GENETIC CODE

## A. Code

Living things depend on proteins for existence. the latter produce enzymes necessary for all chemical reactions. Structural information required to specify the synthesis of any given protein resides in the molecule of DNA which has the spatial configuration of a double helix proposed by Watson and Crick (1953) as given earlier. The linear sequence of bases in DNA constitutes an alphabet (hereditary lettering, generally considered to be of four letters—A, T, G, C) which 'codes' for another linear structure, a protein, written in another alphabet of twenty amino acids. The actual transfer of information is, however, indirect. DNA is a 'template' for the formation of RNAs, which are incorporated into ribosomes and in turn act as templates for protein synthesis. All properties of protein, including its secondary and tertiary structure, are ultimately determined by chromosomal DNA, and all biological properties are in turn determined by the amino acid sequence of the proteins within an organism, either through protein structure or enzymic activity. The term 'coding' implies the relationship between DNA and protein. By coding, the hereditary lettering carried in the four alphabets of DNA is ultimately converted into the protein language composed of twenty alphabets of amino acids.

*Triplet code*: Researches have been carried out by Ochoa, Kornberg, Nirenberg, Brenner, Crick and others to detect the coding ratio, that is, the number of units in one system required to specify one unit in the other system. Certainly no one-to-one correspondence can be observed between nucleotides and amino acids. If each kind of nucleotide specified a single amino acid, only proteins consisting of four amino acids could be constructed. Similarly the correspondence of an amino acid to two nucleotides would give a large number of possibilities but still not enough, only $4^2=16$. If a three-digit code is employed, however, a total of sixty-four kinds of units is established, more than enough to encode twenty amino acids. The surplus forty-four triplets are known as *nonsense codons* and the requisite twenty as *sense codons.* Some of the nonsense triplets may be used as 'punctuation' in a chemical code, designating the beginning or the end of a chemical message.

A number of genes in higher organisms and their viruses appears to be *split*, that is, they have *nonsense* stretches of DNA interspersed within the *sense* DNA. The cell produces a full RNA transcript of this DNA, nonsense and all, and appears to splice out the nonsense sequences before sending out the RNA to the cytoplasm.

Critical information on the nature of coding units, that is, the code is in triplets, was gathered from studies of the mutagenic effect of proflavin on the polynucleotide chain of DNA. Application of proflavin leads to the deletion or duplication of one nucleotide pair or several adjacent pairs. In *E. coli*, addition (+) or deletion (−) of one or two bases respectively causes a drastic effect. The addition or removal of three bases, on the other hand, though causing changes in the behaviour of the organism, yet does not induce a drastic effect and the organism may show a different amino acid pattern. However, concrete evidences of the triplet nature of the codon were obtained later in experiments of Khorana, Brenner and others where synthesised messenger triplets were found to attach specific tRNA amino acid complexes.

The original proposal that the code is in the form of a long sentence, with no punctuation in between, was later modified by conceding the presence of certain periods or stops to account for the functioning of different independent metabolic processes.

*Overlapping code*: In nature, there is always a tendency towards economy. As suggested by Gamow G (1954 *Nature* 173: 318), in his 'overlapping' coding hypothesis, the code is in the form of triplets, but not arranged in a straight chain. It is called overlapping in the regions where a particular nucleotide serves in more than one coding unit. Gamow suggested an overlapping code on the basis of two characteristics:

(i) Distance between two bases in a DNA molecule is 3.4Å.

(ii) In a protein molecule also, the distance between two adjacent amino acids is 3.4Å.

This distance can be explained in cases of monocoding, as well as for overlapping coding but it is quite improbable in a straight chain triplet coding. If the overlapping hypothesis is accepted, alteration of a single base will result in change in at least three amino acids.

*Non-overlapping code*: Works carried out on rat haemoglobin and coat protein of virus showed that a change in one base is always associated with a change in only one amino acid. Evidently the genetic code is non-overlapping.

*Degenerate code*: A convincing amount of evidence indicates that the genetic code is degenerate, i.e., several different triplets code for one amino acid. This is due to the fact that if twenty triplets make sense and the remaining forty-four are nonsense, then in a chromosome, mutation will occur only at limited sites and not throughout its entire length. But the rate of spontaneous mutation as well as the results of induced mutation through X-ray have shown that nearly the entire chromosome site is capable of undergoing mutation. It can be possible only when the code is degenerate. When one triplet is responsible for a number of amino acids it is called *ambiguous* coding. *Unique* code is one, in which every codon is responsible for an amino acid and there is no ambiguity or degeneracy.

*Universality of code*: Since the composition of DNA in all organisms, from viruses to human beings, has been found to be the same, the code was regarded as *universal*. This concept was confirmed by reciprocal experiments in which protein synthesis continued unhampered both when (a) ribosomes of *E. coli* were added to cell-free cultures containing tRNA, mRNA and amino acids of rabbit and (b) mRNA from rabbit was added to a cell-free culture containing tRNA and amino acids from *E. coli*.

## B. Transcription and translation

Whether one or two strands of DNA are active for giving genetic information in coding is a problem. Later experiments have shown that no doubt for the process of replication two strands are necessary, but for gene action and enzyme synthesis, in the control of gene function, only one strand is essential. For mRNA synthesis, the two strands of the double helix separate from each other and mRNA is transcribed, complementary to one of the DNA strands. This process of transcription is mediated through DNA-dependent RNA polymerase. The mRNA synthesis is initiated at the 5′ OH end and the direction of growth is from 5′ to 3′. The RNA polymerase attaches to an initiator site on the structural gene and catalyses mRNA synthesis till a termination site is reached. After transcription mRNA moves into the cytoplasm and a number of ribosomes become attached to it, forming a polyribosome. Following this process, soluble RNA (tRNA) which has two ends (one end possessing three prongs) carries an amino acid at the single pronged base end and ultimately attaches itself at a specific point of mRNA triplet through its 3-pronged base end. Transfer RNAs are specific for specific amino acids which are thus placed in specific points to synthesise the polypeptide. The entire process through which the nucleotide language of mRNA is being translated

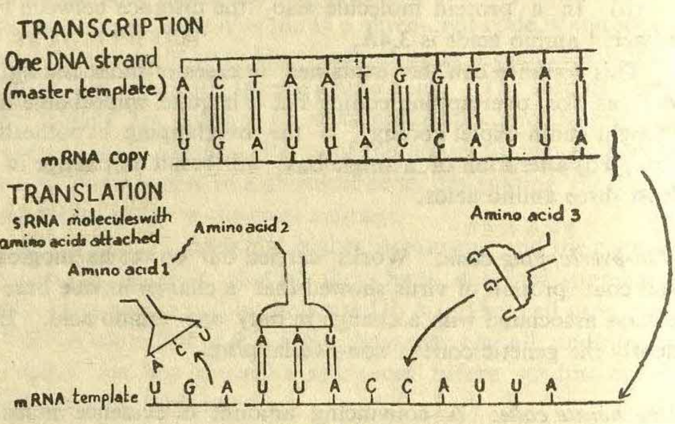


Fig. III : 5.1.

into the amino acid language of polypeptide is known as translation (Fig. III: 5.1).

The enzyme, *DNA gyrase*, essential for bacterial viability, plays a critical role in DNA replication and repair, transcription and recombination. It catalyses the ATP-dependent introduction of negative supercoils of the DNA helix axis itself into circular duplex DNA (Fisher M 1981 *Nature* **294**: 607).

### C. Modulation

In different organs, translation throughout the length of mRNA does not proceed at the same rate. This process, known as modulation, may be responsible for differentiation of organs. If a chromosome is regarded to be a long chain molecule of which genes are merely parts then the process of differentiation will account for switching on or off of certain specific segments of the molecule in different organs. The entire system is controlled by repression and derepression.

### D. Techniques for study

The cell-free system for protein synthesis offers excellent opportunities for exploring the problems of information transfer, the general nature of the genetic code and the nucleotide composition of some of the coding units themselves.

Artificial messages have been of great significance in solving the genetic code. When synthetic RNAs with different known base compositions became available it was possible to test the hypothesis that the coding ratio of nucleotide/amino acid is 3: 1. Ochoa and his collaborators isolated an enzyme known as polynucleotide phosphorylase from microbial cells with properties very different from DNA-dependent RNA polymerase. It had the capacity to catalyse the formation of polyribonucleotides without a 'primer'. By means of this enzyme they were able to synthesise a variety of polyribonucleotides by controlling the nucleotides available to the enzyme and to construct homopolymers containing only uridine (Poly U), or cytidine (Poly C) or mixtures (copolymers) of uridine and cytidine (Poly UC), of uracil and guanine (Poly UG), etc. Copolymers consisting of the same bases but in different ratios of those bases could be synthesised by regulating the ratios in the reaction mixture. In the experiment, an *in vitro* system was used (extract of *E. coli*) which consisted of ribosomes, supernatant containing the activating enzymes, tRNA, ATP, GTP and a synthetic RNA composed only of polyuridylic acid. Under these circumstances the only amino acid incorporated was phenylalanine and polypeptide chains composed entirely of phenylalanine were fabricated. This fundamental discovery demonstrated that the triplet specifying phenylalanine is UUU. The isolation of an enzyme polynucleotide ligase by Khorana, different species of which were specific for joining different nucleotides, led to the synthesis of 64 triplets.

### E. Dictionary of RNA

These experiments received tremendous impetus through the discovery that the addition of trinucleotide to a cell-free system causes the binding of a specific tRNA-amino acid complex to

**TABLE III : 5.1**
**The genetic code**

| *5′-OH Terminal base* | *Middle base* | | | | *3′-OH Terminal base* |
|---|---|---|---|---|---|
| | *U* | *C* | *A* | *G* | |
| | Phe | Ser | Tyr | Cys | U |
| U | Phe | Ser | Tyr | Cys | C |
| | Leu | Ser | CTS | CTS | A |
| | Leu | Ser | CTS | Trp | G |
| | Leu | Pro | His | Arg | U |
| C | Leu | Pro | His | Arg | C |
| | Leu | Pro | Gln | Arg | A |
| | Leu | Pro | Gln | Arg | G |
| | Ile | Thr | Asn | Ser | U |
| A | Ile | Thr | Asn | Ser | C |
| | Ile | Thr | Lys | Arg | A |
| | Met* | Thr | Lys | Arg | G |
| | Val | Ala | Asp | Gly | U |
| G | Val | Ala | Asp | Gly | C |
| | Val | Ala | Glu | Gly | A |
| | Val | Ala | Glu | Gly | G |

CTS = Chain termination signals; * = Chain initiation (Davidson 1972) the ribosome. Once the trinucleotides have been synthesised, the technique is extremely simple. A trinucleotide is tested against 20 tRNA preparations, each loaded with one radioactive amino acid and 19 unlabelled amino acids. Since ribosomes can be separated from unbound tRNA amino acid complex, the ribosome can be identified by taking account of the radioactivity in the membrane after washing it free of unbound radioactivity. The effect observed is small but quite characteristic. The principle is that the radioactive amino acid can be only held on the ribosome, if its specific messenger trinucleotide is present. On the basis of these observations a dictionary of ribonucleic acid code words has been prepared in which each triplet or triplets have been assigned to the code responsible for the synthesis of a particular amino acid or acids (see Table III:5.1).

STRUCTURE OF ADAPTOR RNA: During translation of the genetic code, each amino acid becomes linked to one end of a specific adaptor tRNA molecule with the help of specific amino-acyl activating enzyme. They are then transferred to the ribosome, where the other ends of adaptor RNAs are joined by H-bonds to complementary sites on an mRNA template. The amino acids are thus placed in the proper sequence for polypeptide synthesis. The X-ray diffraction pattern of total adaptor RNA bears a strong resemblance to the diffraction pattern given by DNA indicating that the majority of the molecules is in the double helix configuration. From these observations it was proposed by Holley RW, Apgar J, Everett GA, Madison JT, Marquisee M, Merrill SH, Penswick JR and Zamir A (1965 *Science* **147**: 1462) that a molecule of adaptor RNA normally consists of a single polynucleotide chain with a bend approximately in the middle. The two segments of the chain are wound around each other in a helical manner. The configuration resembles a 'clover leaf' (Fig. III:5.2).

All adaptor RNAs have the terminal sequence adenylic acid-cytidylic acid-cytidylic acid (ACC or CCA) on the 3′ end and guanine or guanilic acid on the other 5 end. The amino acid is attached at the 3′ end, constituting the amino-acyl-tRNA. The combination between a tRNA and its specific amino acid is mediated by an enzyme, the amino-acyl-tRNA synthetase. There are at least twenty different synthetases, each specific for an amino acid and the corresponding tRNA. In making the proposed bend in the middle of adaptor RNA molecule a minimum of three nucleotide residues must be present in the unpaired state. This triplet of unpaired nucleotides in the bend known as the *anticodon* is presumed to react by specific base pairing with the mRNA template. It is capable of recognising the complementary bases in the mRNA and is therefore the most specific end of the tRNA molecule and the one that reads the message. In the other end the unpaired A-C, carrying the amino acid, may give sufficient freedom of movement. In addition to the amino-acyl binding site with the sequence CCA and the codon recognition site (anticodon) binding by hydrogen bonds to the corresponding codon on the mRNA, each tRNA molecule has a ribosome recognition site and a site for the recognition of the specific amino acid—activating enzyme. Smith (JD 1973 *Br Med Bull* **29**: 220) suggests that there is no general synthetase site and that each individual recognition may involve more than one part of the tRNA molecule. The molecule also has several unusual bases, some of which are methylated, including pseudouridine, inosinic acid, methyl guanine and methyl cytosine, amongst others. The first complete tRNA synthesised in 1965 was yeast alanine-tRNA with 77 nucleotides. At present the sequences of several specific tRNAs from different sources are known. In the attachment

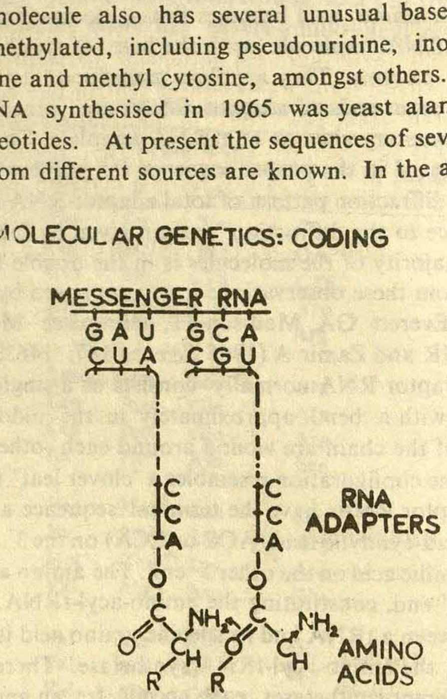


Fig. III : 5.2 Formation of polypeptides through RNA adaptors.

of tRNA to its corresponding amino acid, the aminoacyl tRNA synthetase activates the carboxyl group of the amino acid for covalent bonding with the adenylic acid residue of the tRNA.

POSSIBILITIES FOR THE EVOLUTION OF GENETIC CODE FROM A PRECEDING FORM (Jukes JH 1973 *Nature* **246**: 22): In the primitive forms, the number of amino acids coded might have been more or less than 20. Amino acids might have been added during evolution or may have disappeared. Also arginine has been suggested to have replaced ornithine during the evolution of protein synthesis. The concept that the number of amino acids in earlier codes was less than 20, is favoured by the fact that some of the simpler amino acids, e.g., serine, glycine, alanine have four or more codons and that the codons may be arranged in "quartets", e.g., GCU, GCC, GCA and GCG for *alanine*, in which only the first two bases confer specificity on the amino acid. A code of "quartets" of codons would suffice for not more than 16 amino acids and the present 20 can, therefore, be brought down to a choice of 15. The ancestral code may have had 10 amino acids in which 8 codons were needed for each of the 6 amino acids and 4 codons for each of the 4 amino acids. These 10 amino acids in the ancestral code would be adequate for the synthesis of proteins with reasonably versatile properties. The expansion of the code to 18 amino acids was preceded by tRNA modification in such a way that U and G or G and U pairing was suppressed. This enabled modification of amino acid recognition characteristics by mutation and thus, new amino acids entered into the process of protein synthesis. Further expansion of the code to 20 amino acids then took place, by reassignments of tRNA molecules.

IMPLICATIONS OF MESSENGER ANALYSIS: Two outstanding discoveries have facilitated the isolation of so-called "minor" mRNAs. One of these is the immunological procedure in which antibodies are prepared from the polypeptide chains and from the antibodies later, mRNAs can further be obtained in polysomes. The other method takes advantage of the poly A sequence in mRNA which may be 50-500 residues long at the 3′ terminus in the eukaryotic system. These long poly A sequences are added after transcription. The poly A sequence will specifically interact with poly U or T forming H-bonds found in double stranded nucleic acids. This poly A sequence is specific for mRNAs and therefore, rRNA will not be bound to poly U or poly T attached to inert columns of cellulose or sepharose. The bound mRNAs can further be separated by lowering the ionic strength of the medium, or by formamide treatment which weakens the H-bond interaction.

Another approach has been made in which total mRNA is prepared free of rRNA. Fractions of approximately correct size are taken from a gel or gradient and tested in sensitive cell-free systems.

The discovery of *reverse transcriptase* or RNA-dependent DNA polymerase by Spiegelman and his group, which can make DNA copies of RNA molecules, has helped the study of mRNA. The cDNA copies from the mRNA hybridise to the minus (—) strand of the DNA rather than the coding strand. Most of the mRNAs which have been observed appear to be copies from unique DNA. Only the histone genes have a multiplicity of DNA. It is likely that absence of poly A and gene multiplicity, both of which are attributes of histone mRNA, are related to one another.

There are certain distinct advantages of mRNA analysis and isolation which are outlined below:

(i) Defective genes may be supplied for the possible cure of diseases like phenylketonuria or sickle cell anaemia. The existence of mRNAs for important proteins would be the first step in determining the gene sequence and isolating the gene directly by hybridisation or enzymic synthesis.

(ii) Gene isolation procedure is merely an extension of DNA/RNA hybridisation technology. In SV40 DNA, once a gene specific mRNA is obtained in large quantities, it can be linked to cellulose chemically and will function to anchor its homologous DNA by straightforward hybridisation interaction.

(iii) Gene localisation is another possibility of mRNA analysis. Cells grown from amniotic fluid during early pregnancy may reflect specific enzymic or chromosomal defects which can be correlated with specific human genetic disorders. Accurate chromosome mapping procedure, coupled with *in situ* hybridisation of specific labelled mRNA of cDNA copies, made with reverse transcriptase, should allow a correlation between the structural defects seen and the enzymic disorders. This analysis can be extended to other genes as well.

CRITICISM OF WATSON-CRICK THEORY AS THE CHEMICAL EXPLANATION OF INHERITANCE: Commoner B (1968 *Nature* **220**: 334) cited certain evidences to show that the Watson-Crick theory is unable to explain inheritance in full. According to him, an organism's biological specificity is deduced solely from the nucleotide sequence of the DNA complement, and the organism's capability for self-duplication is derived from the capability of DNA to act as a self-duplicating molecule. Transfer is a one-way process from DNA to mRNA, to protein amino acid sequence, to enzyme specificity to final phenotype.

The evidences deduced by him in support of his criticism include the following:

(i) In the replication experiments in cell-free culture, Speyer and others have discovered that if a particular mutation with altered DNA-polymerase is used in *E. coli*, the DNA double helix is not replicated with absolute fidelity and mutations often result. Unlike the normal polymerase, the mutant enzyme can control GC replication more rigorously than A-T replication. Hence, in the replication of DNA it is inferred that nucleotide sequence of the new DNA molecule is determined jointly by the parental molecule and by the DNA polymerase.

(ii) Protein is synthesised in a complex process involving ribosomes, mRNAs and tRNAs to which specific amino acids are attached and synthetase enzymes. The experiments of Nirenberg and others imply that in certain cases, a given codon yields different binding capacities when tested against amino acid tRNA complexes originating from different species (*E. coli*, guinea pig liver or *Xenopus* liver). Thus, their data show that the protein-amino acid sequence may be governed by specificity derived from both mRNA and the amino acid-tRNA complex, mediated by tRNA amino acid synthetases.

(iii) The next question involves the protein synthetases which link the successive amino acids to form the protein-polypeptide chain. Evidences so far obtained show that two synthetases originating in different species give rise to quite different degrees of incorporation of the same amino acid into protein.

Apparently the biochemical specificity of the protein must be derived in part from the biochemical specificity of the synthetase which synthesises it.

(iv) A possible contribution to protein specificity by ribosomes has been uncovered. When an *E. coli* strain with a particular genome is provided with two alternative mutations, both of which can be shown to affect the ribosomes, two different influences of the original phenotype are observed. It is implied therefore that the different ribosomal mutants may permit qualitatively different types of translation of the same codon.

Summarising the situation, the Watson-Crick theory proposes a system of specificity transfer which operates in one direction, from DNA through protein enzymes, to the biological phenotype. The evidences discussed by Commoner show that the systems which govern the specificity of both protein synthesis and DNA synthesis, are fundamentally similar. Biochemical specificity embodied in DNA-nucleotide sequence arises partly from pre-existing DNA and partly from pre-existing proteins. At the same time, biochemical specificity embodied in the amino acid sequence of protein, arises partly from protein and partly from DNA. Commoner criticises Watson-Crick theory mainly on the basis of its implications. According to him, in gene therapy, as suggested by Sinsheimer, the multimolecular system is to be taken into account. Mere chemical manipulation of DNA molecule may not lead to significant results in the cure of diseases. In any case, even though the control of replication, transcription and translation may involve several factors, yet the ultimate specificity of the different factors is governed at the level of DNA double helix.

DISPENSIBLE AND INDISPENSIBLE GENE: Biochemical genetics of *Neurospora* led to the discovery of a class of mutations referred to as *dispensible.* The wild type alleles of these genes determine the structure of a product such as an enzyme which is non-essential, i.e., the enzyme can be dispensed with, since it catalyses some steps in the synthesis of a low molecular weight compound, such as soluble vitamin or amino acid which can be readily supplied to the organism by adding to the nutrient medium. Certain gene functions on the other hand are indispensible and cannot be compensated for by supplementing the minimum medium. That means mutation of such an indispensible gene would be lethal to the micro-organism even when a medium enriched with every conceivable nutrient is used.

A method for finding out indispensible mutation was devised by Atwood et al. The mutants are referred to as heterokaryon mutants because the mutant nuclei exist in symbiosis in the same cell with nuclei bearing normal genes, performing indispensible functions. The heterokaryon therefore resembles a diploid organism, heterozygous for a recessive lethal. Out of such cultures conidia may be produced containing only the mutant nucleus which will not grow in the complete medium. It has been shown that majority of mutants in *Neurospora crassa* belong to the indispensible category. There may be a third class of genes which are so essential that once having undergone mutation they cannot survive in heterokaryons. These would be dominant lethals in effect and are difficult to detect.

## F. Problem of differentiation

Differentiation is the developmental process which leads to the differential functioning of different parts of an organism. In unicellular organisms obviously a single cell or its parts are affected. But in multicellular organisms, different organs acquire different function and structure during the course of development. These variations are the consequences of gene products which may differ in quantity and quality in different organs or even in the efficiency of their functioning. The process is gene controlled and phasic and involves a series of chemical reactions for the ultimate manifestation of a tissue or an organ. Since in eukaryotes, the chromosomal complement of all tissues is similar, the basis of differentiation requires an explanation different from the prokaryotes.

The progress of differentiation during development follows a system of repression and derepression of successive genes as explained in the *operon concept* in the next chapter. In case more than one gene are involved in a particular process, these may be located on different chromosomes. Thus an interchromosomal control may be visualised for a common inducer or signalling system operating as a multiple operon system.

Changes in the chromosome structure and its components have been recorded in the different phases of eukaryotic development and growth. For example, protamine in sperm cells is replaced by histones in somatic cells. The arginine and lysine components are again variable in different organs. The chromosomes packaged for transmission in the germ cells are thus not identical with those in the somatic cells. The eukaryotic pattern of differentiation differs from the prokaryotic one in the interchromosomal control and complexity in the chromosome structure—both physical and chemical.

A baffling aspect was that the nuclei in differentiated tissues were apparently inactive as regards cell division. This nuclear inactivity could not be reconciled with the continuous gene activity required for differentiation. This problem was resolved from the observation that nuclei in differentiated tissues often exhibited partial endomitosis in which entire chromosomes or segments existed in a polytenic condition.

Endomitotic replication of chromonemata in differentiated nuclei was observed by Huskins and later workers, including the author. This was visualised because gene action responsible for differentiation was considered to be dependent on chromonemal replication.

Later discoveries show, however, that transcription, responsible for gene action (enzyme synthesis), is entirely independent of DNA replication. So there is no apparent necessity of endomitotic replication to bring about differentiation.

But endomitotic replication is an observational fact along with the existence of diploid nuclei in adult tissue. The existence of both diploid and polytenic nuclei in differentiated cells can be explained by assuming that a *molecular life* is involved in all metabolism. It was therefore suggested that a DNA thread may transcribe mRNA only for a limited period, after which a new thread must be developed by replication to carry on the process of differentiation. So in adult tissues the different sizes of nuclei may represent different ages depending on the number of endomitoses they have undergone.

Improvements in DNA densitometric measurements have given evidences in support of this concept. Endomitotic nuclei in different organs of the same organism give different DNA values which are above the usual 2C value and increase with progressive differentiation (Sharma AK 1975 *Nucleus* **18**: 93).

## G. Repeated DNA

A feature of eukaryotic DNA which is of great interest is the presence of DNA sequences that occur many times in such cells. Unlike bacterial DNA which is thought to consist of a linear array of unique sequences, the DNA of each eukaryotic cell contains many copies of some of its DNA sequences. Moreover, there is a large variation in the amount of DNA in the cells of some closely related species (e.g. someamphibian species have 10-20 times DNA as compared to the other closely related species). The organisation of repeated sequences is believed to be related to the control genes (Crick FHC 1971 *Nature* **234**: 25). Through denaturation and renaturation of mouse DNA, some DNA was found to consist of families of repeated sequences (Britten RJ and Kohne DE 1968 *Science* **161**: 529). Ten per cent was found to be of a highly repetitive nature ($1 \times 10^6$ copies), 15% of families of related sequences (1 to 100 thousand copies) and 70% of unique sequences. The repetitive sequences appeared as a light satellite peak rich in AT regions, separate from the main peak DNA when run in a caesium chloride gradient centrifugation. With investigations on different groups of organisms, more families of repetitive sequences are being found. In the human system, 30-35% of the genome is repetitive (see *Modern aspects of Genetics* 1973 ed RAZ Pfeiffer Schattauer Stuttgart).

Though several models have been proposed which differ from one another in details, it has been more or less agreed upon that repeated and unique DNA sequences of many organisms are interspersed in specific patterns. These patterns may be common to all eukaryotic DNA. Britten studied repeated sequences with his technique of reassociation. He sheared DNA into short fragments and observed the reassociation rates as a function of fragment length. According to him, the repeated segments reanneal very quickly.

The percentage of DNA responsible for coding protein was determined through hybridisation with mRNA for proteins in mouse, duck, Dictyostelium, etc. These results show that most proteins are coded by unique DNA sequences with the exception of histone. The sequences that code for histones are repeated 400-1200 times depending upon the species, and the repeated sequences are clustered in the molecule. Such sequences of repeated DNA have been shown to be organised in specific patterns along with unique sequences which are important for genetic regulation.

About 50% of sea urchin and *Xenopus* DNAs consist of closely interspersed repetitive and unique sequences. The repetitive sequences are composed of copies of about 200-400 nucleotides and unique sequences of about 650-900 nucleotides. The remainder of the DNA consists of long unique sequences interspersed with short repetitive sequences about which very little is known (Lee CS and Thomas CA 1973 *J Molec biol* **27**: 25).

Thomas developed a method based on the assumption that if DNA containing densely clustered repeated sequences is randomly broken, it is likely that many fragments, containing complementary nucleotide sequences at both fragment ends, may be present. Such fragments can form rings which may even be observed under the electron microscope. The fact that long fragments formed few rings in *Drosophila* salivary gland chromosomes, indicates that repeated sequences are clustered in segments which are rather small. According to Thomas, in *Drosophila*, the repeated sequences are interspersed by an equal amount of non-repeated sequences. Several authors have noticed (Burke-Judd) that each band contains enough DNA to code for 30 proteins and therefore only 1/30th of *Drosophila* DNA need consist of genes.

This experiment implies that protein coding information is repeated many times in a band. This model of chromosome structure differs from the model of Britten and Davidson who believe that repetitive sequence consists of 200-400 nucleotides and the longer unique sequences are the genes.

The controversy between these two views was resolved by Bonner and Wu. They broke DNA following similar techniques, to study the distribution of repeated sequences. The fragments were allowed to reassociate under conditions that favoured reassociation of repeated sequences. Most repeated segments were separated by 750 nucleotides although some were separated by sequences as long as 3000 nucleotides. On the basis of these results, they proposed that each band of *Drosophila* chromosome consists of 30-35 unique sequences which are separated by short repeated sequences. Moreover, several repeated sequences in a band are alike. Their idea, therefore, suggested that each band is also associated with a single family of repeated sequences. Bonner believed that his model of *Drosophila* DNA could be extended to rat DNA. He proposed that rat DNA is organised in regions in which the same repetitive sequence separates various unique sequences in a region (see also Fig. II: 3.2).

CHAPTER III: 6

# FUNCTIONING OF GENE

## A. Operon concept

Jacob F and Monod J (1961 *Cold Spring Harb Symp Quant Biol* **26**: 183 and *J Mol Biol* **3**: 318) formulated the concept of *operon*—unit of operation in a chromosome. They observed that the three enzymes—β-galactosidase, acetylase and permease, can be induced to *appear* in the bacterium *E. coli* by the addition of an inducer in the medium. These enzymes act in a coordinated manner in heredity. A mutation was later discovered in which all these three enzymes were found to be produced in a large amount in the absence of an inducer. All of them are affected by genes that map within the *lac* locus of *E. coli* in the sequence *z* (β-galactosidase)—*y* (permease)—*a* (acetylase). In the former case, where the enzymes were induced, they were termed *inducible* as compared to the latter where the property is constitutive. Their behaviour in inheritance shows a genetic control. Such constitutive mutations were mapped at one end of the *lac* locus not far from *z*.

On the basis of these observations supplemented by similar behaviour in various other bacteria, the operon concept was postulated. It visualises that in a chromosome the structural genes are responsible for the synthesis of enzymes e.g. *z*, *y* and *a*. They behave in a coordinated way and are controlled by an operator gene. Its activity, in turn, is inhibited by a repressor gene which may not necessarily be located adjacent to it under normal conditions. The repressor produces an inhibitory substance which checks the activity of the operator so that the structural genes are unable to function. Following a mutation, the repressor may be inactivated and as such the operator will be able to operate freely.

The inducible substance or inducer may directly bind with the repressor or regulator gene at the DNA level or may combine with its product so that it cannot reach the operator. It is yet to be determined precisely except in *lac* operon whether the latter duplicates during the production of mRNA.

The entire complex is termed an *operon*. An operon may be monocistronic or polycistronic depending on the number of structural genes (*cistron*, acting in a coordinated way). The operon complex controls the synthesis of m-RNA by RNA polymerase. The presence of a repressor at the operator locus in the *lac* system thus blocks transcription of the operon by RNA polymerase. On the other hand, an appropriate inducer removes the repressor, thus permitting transcription. Constitutive enzyme synthesis, which requires continued transcription of structural genes uninhibited by repressor, may occur through a mutation (a) either of the regulator gene to produce an inactive repressor unable to bind to the operator, (b) or of the operator making it incapable of binding the repressor. The simplicity of the operon concept, as a set of structural genes controlled by an adjacent site, has led to it becoming one of the central concepts of genetics. The most interesting part of this concept is that under wild type or normal condition the control is negative and the mutation shows positive behaviour in a

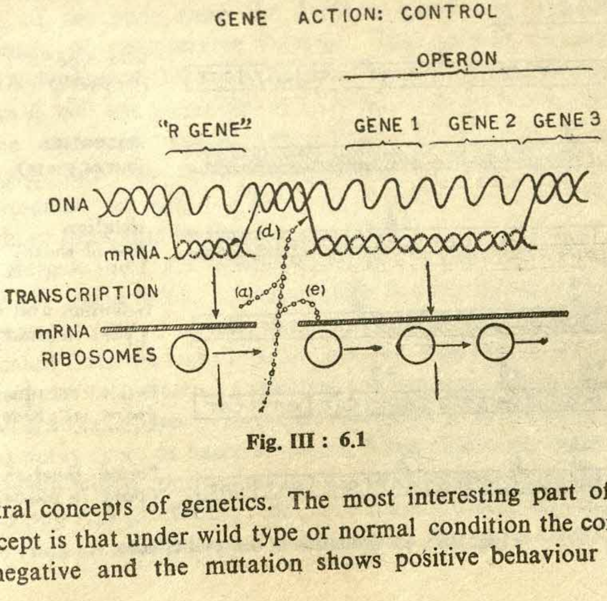


Fig. III : 6.1

prokaryotic system (Fig. III : 6.1).

The way through which mutation in operator or regulator could be determined remained problematic for some years. At the diploid level, following sexduction or conjugation in which DNA is transferred directly from one bacterium to another by pairing, there is a convenient way through which the mutation in operator or regulator can be visualised. According to Jacob, the repressors can be compared with two transmitters emitting inhibiting signals to two rooms which are connected through a connecting door. The two rooms on the other hand have two receivers or operator genes, placed at the two exits leading to two separate chambers in which the structural genes are located. The purpose of considering the two rooms as being connected is simply because of the fact that the two genophores are embedded in a common background.

In the first possibility where mutation may involve the regulator gene, one of the transmitters will be ineffective whereas the other one will be emitting the inhibitory signal to be taken up by the two receivers or operators. This would result in the continuation of the normal repressing activity and as such the mutation would be recessive or have no effect at the diploid

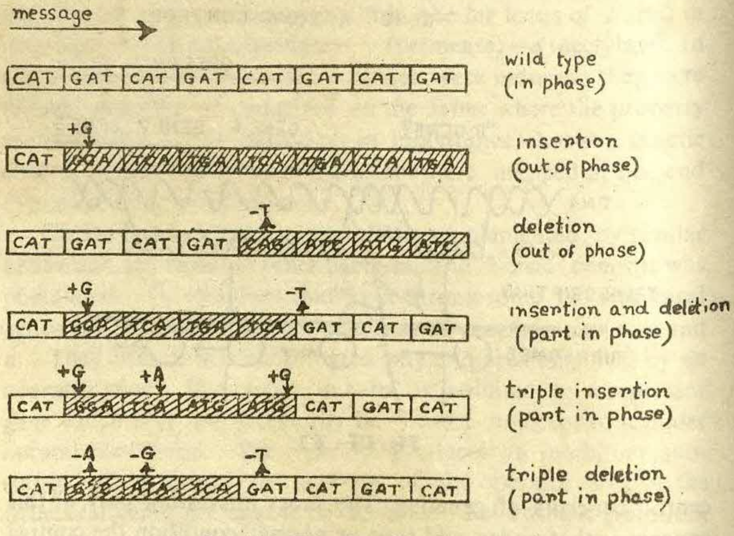


Fig. III : 6.2 Mutation at the level of code.

level. On the other hand if the mutation is in the operator, one of the receivers will be ineffective and as such the structural genes lying in the closed chamber would not receive the inhibiting signal. The result would be that they would be functional. Therefore a mutation in an operator level will immediately lead to its visible expression and the effect would be positive at the diploid level. The repressor and operator mutations can thus be differentiated (Fig. III : 6.2).

INITIATION OF POLYRIBONUCLEOTIDE CHAIN DURING TRANSCRIPTION: Polyribonucleotides may be synthesised by any one of three different enzymes:

(i) by the action of DNA-dependent RNA-polymerase (nucleotidyl transferase) during transcription;

(ii) by the action of RNA-dependent RNA-polymerase, as for example in cells infected with RNA viruses; and

(iii) by the action of polynucleotide phosphorylase—an enzyme isolated by Ochoa and his group from *Azotobacter vinelandii*, which catalyses the synthesis of high molecular weight polyribonucleotides from nucleoside 5′-diphosphates with release of orthophosphate.

The initiation of the polynucleotide chain during transcription of the code from the DNA to a particular mRNA is a problem of considerable interest. The enzyme concerned is DNA-dependent RNA-polymerase. The holoenzyme as obtained from *E. coli* has a number of subunits, namely two $\alpha$ chains of same molecular weight, two $\beta$ chains of different molecular weights and one molecule of sigma ($\sigma$) factor and may be represented as $\alpha_2\beta\beta'\sigma$. The core enzyme is the holoenzyme without the sigma factor.

Burgess and his colleagues (1969 *Nature* **221**: 43) showed that the sigma factor is responsible for the initiation of RNA synthesis. When synthesis of RNA has begun, this factor is released from the transcription complex and can be used again. It appears to operate in selecting the genes at which transcription is to take place. In the transcription of DNA from *E. coli* and many types of bacteriophages, RNA chains are often found to begin with a purine nucleoside triphosphate like ppp $G_pY_p$ and ppp $A_pY_p$. Within the core enzyme alone, less than half the ends are of this type. When sigma factor is added, more than 70% of the chains begin in the same way. Apparently in the absence of the sigma factor, the core enzyme initiates RNA synthesis at random points. When it is present, on the other hand, RNA polymerase transcribes only the minus strand of DNA at specific initiation points, the promoters in the DNA template. It then undergoes a conformational change that allows ribonucleoside 5′-triphosphates to be attached at the binding sites with the formation of the first internucleotide linkage in the RNA chain to be produced. Only one type of sigma factor is at present known and it forms an integral part of RNA polymerase. It possibly causes the polymerase to bind to promoter sites, and not elsewhere, by decreasing the non-specific binding of the core enzyme to DNA. After forging the initial internucleotide at the 5′ terminus, the factor is released, reducing the affinity of the polymerase for the promoter site and allowing the core enzyme to move along the DNA template, with the corresponding synthesis of a RNA strand, as observed under the electron microscope as well. The action of sigma factor is believed to be supplemented by several other protein factors.

As a first step, the RNA polymerase forms an enzyme-DNA complex which becomes associated with a purine ribonucleoside 5′ triphosphate. The new chain grows by the subsequent addition of ribonucleotides to the free 3′-hydroxyl group. Synthesis proceeds from the 5′ end to the 3′ end of the RNA molecule. The nature of the initiation point has not been fully clarified. The nucleoside residue at the 5′ end of the chain always contains a purine base. Clusters of pyrimide residues on the DNA strand being transcribed have been suggested to form the initiation point. The RNA chain, after transcription, separates from the DNA. In *E. coli*, it associates immediately with ribosomes so that polysomes may start forming before the completion of transcription. A protein factor termed the rho (ρ) factor causes the RNA polymerase to recognise specific termination signals, terminating the RNA chain and releasing it from DNA template.

In mammalian cells, the RNA polymerase is very tightly bound to the deoxyribonucleoprotein of the nucleus. DNA may serve as a template for the production of as much as a 60-fold yield in terms of RNA

The different types of RNA synthesised by the polymerases of the cell nucleus are:

(i) nuclear precursor of rRNA, i.e., 45S RNA
(ii) precursor of tRNA
(iii) heterodisperse DNA-like RNA (Hn RNA)
(iv) mRNA
(v) miscellaneous low weight RNAs.

Precursor of rRNA is synthesised from the DNA in the nucleolar organiser region, as discussed elsewhere. A very small proportion (about 0.025%) of the DNA of the cell contains base sequences complementary to tRNA.

INITIATION AND TERMINATION OF PROTEIN CHAIN: In the translation of a mRNA, the starting point for the synthesis of protein must be a definite one. It was initially thought that the first codon calls forth the delivery of protein and no special starting signal was required. Later works have shown that the situation is quite different. In *E. coli*, methionine-tRNA complex was tagged with radioactive sulphur and after extraction from the growing cell, following digestion with pancreatic ribonuclease, formylated methionine complex was detected; together with non-formylated methionine adenosine complex. The aldehyde group replaced one hydrogen of the amino radical. This experiment proved that the growing cells contain a high proportion of methionine-tRNA. No peptide bond can be formed by the amino group if it is formylated.

Since the amino group forms the front end of the polypeptide, formylated methionine must constitute the initial unit of the molecule. The donor of the formyl group was found to be tetrahydrofolic acid. Methionyl-tRNA-transformylase catalyses the transfer of the formyl-residue from N=10-formyltetrahydrofolate to the amino group of the methionyl residue attached to tRNA. Later, several synthetic mRNAs were prepared as in Brenner's experiments, but in these cases, no other amino acid could be formylated. It was noticed that the triplets AUG or UUG resulted in the synthesis of polypeptide with methionine as the starting point. This occurs, however, only in those cases where no formylable version of tRNA is available.

In a number of *E. coli* cells, the front end of the molecule was found to be alanine or very rarely, serine, and not necessarily methionine. In extracted RNA from bacteriophages in cell-free system formylated methionine is seen to be the first amino acid, followed by alanine. It is therefore, claimed that in living systems, methionine is chopped off by the enzyme after initiation. That initiation is always by methionine is established, but it is not clear yet as to why alanine or serine comes next.

A good deal of work has been carried out on the codon responsible for formylated methionine. AUG has been found to be common for both formylated and normal varieties of methionine; but GUG codes only for the formylated variety. This has been confirmed in Khorana's experiments. If AUG is in the beginning of the code, it starts the synthesis of polypeptide, but if it is in an intermediate position, it codes for only a methionine. GUG on the other hand, codes for methionine at the starting point, but internally it codes for valine. Therefore, the codon in itself may not constitute the entire message for the initiation of protein. It is likely that messages are only approximations which may accelerate or stimulate the cell machinery in a certain direction.

Further experiments have shown that the tRNA responsible for chain initiation has a special structure. In ribosomes, two sites have so far been worked out; (i) amino acid site, that is, the point of attachment of tRNA-amino acid complex to the mRNA on the ribosome, and (ii) the peptide site where the amino acid must be held together to form the peptide bond. The tRNA responsible for chain initiation is supposed to have a molecular configuration which can fit itself on the peptide site of the ribosome. Binding studies have suggested that initiation of protein synthesis starts with the formation of a complex consisting of mRNA, 30S ribosomes and the initiator formyl methionyl tRNA (f Met. tRNA) in the presence of GTP and initiation factors. 50S ribosomes are then attached to the complex. The f Met. tRNA moves from the amino-acyl tRNA site (A) to the peptidyl tRNA site (P)—a phenomenon known as translocation, involving a movement of mRNA relative to the ribosomes. The vacant site on the ribosome is then occupied by that amino-acyl tRNA which is specific for the codon next to AUG. The peptide bond is then formed with the help of the enzyme peptidyl transferase located on the 50S subunit in the presence of mono and divalent cations (Mg and K or $NH_4$). The dipeptidyl tRNA is then translocated from the A to P site while the deacetylated f Met. tRNA is released from the P site. The process is repeated for successive tRNA's corresponding to the successive codons. When synthesis reaches a nonsense codon the protein chain is terminated. Two release factors, $R_1$, specific for the nonsense codons UAA or UAG and $R_2$, specific for UAA and UGA are involved in the termination of protein chain and release of polypeptide.

REPAIR OF GENETIC SEGMENTS: The ability of cells to repair defects in their DNA may well have been a significant factor in biological evolution. In the molecular architecture of DNA, certain features can be observed which facilitate both recognition of damage and its repair. Since the two strands of DNA helix are complementary to each other the information in a damaged part of DNA can easily be retrieved from its corresponding complementary strand and the cell can use the undamaged DNA as a template for the reconstruction of a new segment. Almost all cells possess enzymes that can repair damaged DNA and thus help to maintain normal function and viability. Several types of repair mechanism are known (for details, see molecular basis of mutation, also Table III:6.1). Photoreactivation involves a single DNA strand only. Mechanisms dependent upon the complementary strand include pyrimidine dimer excision, excision of alkylated products from DNA, of X-ray and γ-ray products from DNA and repair of X-ray induced chain breaks. For the repair of two-strand damage, different mechanisms are postulated such as genetic exchanges with homologous DNA molecules. The combined action of multiple repair systems is generally much more efficient than single methods acting alone.

The simplest example of repair can be found in the process called photoreactivation. Although hints of its existence can be traced back to 1904, photoreactivation was not adequately appreciated until Kelner rediscovered the effect in 1948. The number of soil organisms (Actinomycetes) that survived large dosages of UV radiation could be increased several hundred thousand times if the irradiated bacteria were subsequently exposed to an intense source of visible light. He concluded that

TABLE III : 6.1

A summary of four DNA repair mechanisms

| *Excision repair* | *Postreplication repair* | *Photoreactivation* | *SOS repair* |
|---|---|---|---|
| Damaged DNA areas excised and new sequences resynthesised in gaps<br>Multiple enzymatic functions involved in repair; including recognition of damaged sites, nuclease base removal, polymerase base replacement, and ligase reattachment of DNA ends<br>Involved in human DNA repair<br>Broad range of DNA lesions repaired, including altered bases, larger lesions, or cross-linking of DNA | Only occurs during DNA replication<br>Enzymatic resynthesis is involved<br>Involved in human DNA repair activity<br>Repair involves lesions which result in replication gaps such as altered bases or larger [single-stranded lesions<br>May involve recombinational events | Specific for the repair of ultraviolet light-induced damage (pyrimidine dimerisation)<br>Enzymatic action in the presence of visible light opens the dimers to monomers<br>Not easily demonstrated in human cells<br>Little or no relationship to repair of chemical damage | Appears to be confined to prokaryotic organisms such as bacteria; has little or no effect in human DNA repair.<br>The repair system is not normally present, but is induced by DNA damage that cannot be repaired by the normal excision or postreplication repair processes.<br>Often leads to error-prone repair among surviving cells. |

UV radiation exerted its principal effect on the nucleic acid of the cell.

The germicidal action of UV radiation is due chiefly to the formation of two unwanted chemical bonds between pyrimidine bases that are adjacent to each other on one strand of the DNA molecule, thymine dimers being most frequent. Consequently the bacteria whose DNA is rich in thymine tend to be more sensitive to UV radiation than those whose DNA is not. Photoreactivation involves the action of an enzyme that is selectively bound to DNA which has been irradiated with UV. When this enzyme is activated by visible light (which simply serves as a source of energy), it cleaves the pyrimidine dimers, thereby restoring the two bases to their original form.

Dark reactivation of repair process can be turned off genetically by finding mutant strains of bacteria that lack the repair capabilities of original radiation resistant strains. 'B/r' strain of *E. coli* first isolated in 1946 by Witkin was found to be UV resistant. The first radiation sensitive mutants of the strain known as $Bs_{-1}$ were discovered in 1958 by Ruthhill and their sensitivity to radiation was regarded as due to the malfunction of a particular enzyme that enabled resistant bacteria to repair damage. Then the question arises whether the same enzyme operates in both photoreactivation and dark repair process or resistant cells can somehow bypass the dimers during replication of DNA and leave them permanently present, although harmless in their descendent molecules.

The actual mechanism is even more elegant than either of these possibilities. It exploits the redundancy inherent in the genetic message. The radiation resistant strains of bacteria possess several enzymes that operate sequentially in removing the dimers and replacing the defective bases with the proper complements of the bases in the adjacent 'good strand'. The importance of polynucleotide kinase action was realised in order to provide base for polynucleotide ligase activity. The role of ligase is to re-establish continuity by joining a newly synthesised segment with a pre-existing one through a phosphodiester bond.

Steps that seem to be associated with repair of DNA and that have been postulated to be an integral part of repair mechanism are excision, breakdown of DNA, repair replication and rejoining of strands.

It is evident that the first step in the repair process must involve the recognition of the damaged region in the DNA. A rapid communication system is then needed to 'inform' the normal growing point in the DNA of impending disaster so as to halt replication until repairs have been made. Two possibilities have been suggested, although there is at present no experimental evidence for either one:

(i) The clamping of the recognition enzyme to the damaged region of DNA may provide a physical constraint to the rotation of the molecule as necessary for the unwinding prior to replication.

(ii) Distortions in the secondary structure of DNA (or single strand breaks) may alter the pattern of "breathing" of the DNA. A pyrimidine dimer must represent a "permanent node" in the breathing pattern which could result in a "message" being transmitted to the normal growing point to halt replication.

The excision of dimers was first demonstrated by Setlow and Carrier and was confirmed by Boyce and Howard-Flanders. In their studies cultures of UV resistant and UV sensitive bacteria were grown separately in the presence of radioactive thymine, which was thereupon incorporated in the newly synthesised DNA. The cells were then exposed to UV radiation. After about 30 minutes they were broken open so that the fate of the labelled thymine could be traced. In the UV-sensitive strains all the thymine that had been incorporated into DNA was associated with the intact DNA molecule. Therefore any thymine dimers formed by UV radiation remained within the DNA. In the UV-resistant strains, however, dimers originally formed in the DNA were found to be associated with small molecular fragments consisting of no more than three bases each (thymine dimers were distinguished from individual bases or combination of bases by paper chromatography).

This repair or patching of gaps was directly indicated by 5-bromouracil incorporation into DNA after UV irradiation of the resistant strain of *E. coli* by placing them in a medium containing 5-bromouracil instead of thymine. Before normal rapid DNA synthesis was resumed, the bacterial DNA was isolated and fractionated by equilibrium centrifugation in caesium chloride. Much of the incorporated bromouracil was found at a density corresponding to normal DNA and a hybrid of one normal strand and one heavy strand. A further fragmentation of DNA by sonication, which reduced the molecular weight of the DNA fragments, still left the incorporated label at the normal density. Denaturation of DNA followed by caesium chloride centrifugation also resulted in the same amount ofbromouracil being observed in normal densities. This result indicates that unlike semiconservative replication the bromouracil is incorporated at random into many short single strand segments of the *E. coli* chromosome.

The detailed sequence of events involved in the process of repair has been suggested through two models. In the first one i.e., *cut and patch* scheme, it is postulated that an enzyme excises a short single stranded segment of the damaged DNA. The resulting gap is enlarged by further enzyme attack and then the missing bases are replaced by repair replication in the genetically correct sequence.

In the *patch and cut* scheme the process is assumed to be initiated by a single incision that cuts the strand of DNA near the defective base. Repair replication begins immediately at this point and is accompanied by *peeling off* of the defective strand as the new bases are inserted. This scheme is attractive because it could conceivably be carried out by a single enzyme complex or particle that moves in one direction along the DNA molecule, repairing defects as it goes. It does not involve the introduction of long single strand regions into the DNA molecule while the repair is taking place. Both models are undoubtedly over-simplifications of the actual molecular events inside the single cell but the second one appears to be more reasonable.

Evidences for excision repair have also been obtained from *Bacillus subtilis*, *Micrococcus radiodurans*, Pleuropneumonia-like organisms. Most attempts to demonstrate excision of UV induced pyrimidine dimers from DNA in mammalian cells have so far been unsuccessful, but indirect evidences suggest that repair replication may occur in human cell lines (RA, $RAX_{10}$ and HeLa) in tissue culture. Photoreaction activity has been observed in extracts of blue green algal cells.

Later works show the presence of multiple repair mechanisms, sometimes more than one, for a particular job. Recombination appears to play an important role, especially where both strands of a DNA molecule are damaged at about the same level. The mechanisms for by-passing damage in the template DNA molecules in post-replication repair are very efficient in animal cells, though not yet fully explored. Certain rare hereditary disorders in man appear to be caused by defects in repair.

### B. Molecular basis of mutation

The molecular basis of mutation could only be explained after the discovery of the double helix configuration of DNA and the deciphering of the genetic code (see also Sharma A 1984 Environment chemical mutagenesis *Persp Rep Ser 6*, Golden Jubilee Publ, Indian National Science Academy, New Delhi).

The lesions induced in the double helix during mutational events may involve a large number of base pairs (*macrolesions*), a few base pairs (*microlesions*) or a single base pair (*point mutation*). The macrolesions, if sufficiently large, may be associated with visible chromosomal rearrangements.

As with chromosomal alterations, described in detail in a separate chapter, macrolesions may involve quantitative changes in base pairs (*deletion* and *duplication*) or qualitative rearrangements (*inversion* and *translocation*). Quantitative alterations may be detected by hybridising the DNA extracted from the normal and mutated forms. In both cases, one strand forms a loop. Radioactive labelling of the DNA from either the parent or the mutated form can differentiate between deletion and duplication.

Microlesions or point mutations cannot be identified cytologically. The different types include :

(a) *base pair substitution* involving a single base pair. A substitution of a purine by a purine or a pyrimidine by a pyrimidine maintains the purine orientation and the process is known as *transition*. When the orientation is reversed through the substitution of a purine by pyrimidine or *vice versa*, the phenomenon is referred to as *transversion*,

(b) *frameshift mutation* involves a small number of base pairs, changing the type of amino acid produced, *Transition* mutations may be caused by:

(a) Tautomerism of bases: four kinds of bases can exist in tautomeric forms through proton shift. Thus they can mispair with non-complementary bases, leading to spontaneous mutations.

(b) Base pair analogues, when incorporated, can replace the base and hamper replication. For example, thymine analogues like 5-bromouridine can replace thymine during DNA synthesis.

(c) Certain other chemicals which may behave like base analogues. The more specific ones include hydroxylamine (acts on all bases but only on cytosine at low pH and high concentrations); alkylating agents acting on guanine preferentially, etc.

*Transversion* may result from deletion of a purine base by a chemical (ethyl ethane sulphonate) and its replacement during the next replication cycle by a pyrimidine.

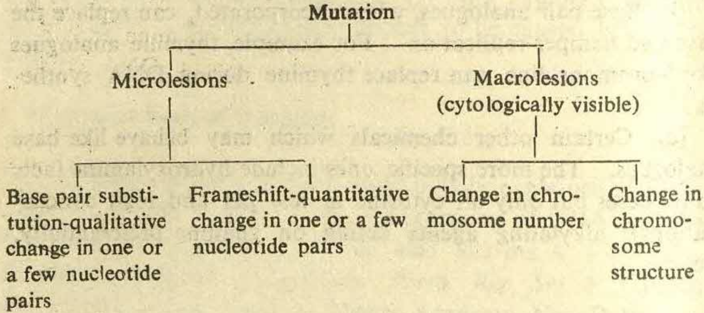
*Frameshift mutation* involves a shift in the frame of the reading of genetic code and is caused by the addition or removal of one or two bases. A shift will occur from the deleted site onwards or from the addition onwards so that the entire code will be read wrongly and lead to the production of altered amino acids. Change of three bases will alter the entire segment.

When grown with a large population of normal cells, mutants may revert to the original type. A reversion at the site of mutation, bringing back the original DNA configuration is known as *true reversion*. A change at a different site which nullifies the mutational effect is called *partial* or *pseudoreversion* while a change at different site other than the mutational one is referred to as *suppression*. Frameshift mutations, when induced by uv rays, form thymine dimers, leading to their deletion.

The expression of mutations is greatly masked by other biological phenomena like the DNA repair mechanism as described elsewhere. In competition with normal cells, usually mutated ones are at a disadvantage and their survival depends upon several factors like the stage of division, the degree of chromosomal coiling, the ploidy level and the chromosomal region involved.

**TABLE III : 6.2**

**Classification of molecular changes in DNA due to mutation**


Mutation
Microlesions
Macrolesions (cytologically visible)
Base pair substitution-qualitative change in one or a few nucleotide pairs
Frameshift-quantitative change in one or a few nucleotide pairs
Change in chromosome number
Change in chromosome structure

ACTION OF CHEMICAL MUTAGENS ON GENETICAL SYSTEM may be:

**Direct**

(a) *On resting DNA* by (i) deamination of bases; (ii) alkylation of bases; (iii) crosslinking of DNA chains; (iv) deletion and translocation of DNA molecules.

(b) *On replicating DNA* by (i) incorporation of base analogues; (ii) intercalation between bases; (iii) interaction with membranes; (iv) interaction with enzymes.

**Indirect** through :

(i) mistakes in DNA replicating enzymes; (ii) mistakes in DNA repair enzymes; (iii) events during recombination; (iv) stimulation or suppression of DNA repair processes; and (v) anomalous chromosome behaviour.

These changes can be produced by radiations and a variety of chemical mutagens and their expression depends on the extent of DNA repair.

REPAIR SYSTEMS AND EXPRESSION OF MUTATION

Biological systems possess certain enzymatic mechanisms which enable them to repair or eliminate genetic damage.

Mutations may result from *imperfect DNA replication*, *unrepaired DNA damage* and *error-prone DNA repair*. If the repair is correct, there is no effect on the system. Unrepaired or wrongly repaired DNA leads to changes in such physiological processes as cell growth, cell division, transcription, DNA replication, gene control, resulting in cell death and malignancy in addition to mutation. DNA damage has been associated with a number of disease conditions including cancer, senescence, birth defects and so on. Cells unable to repair DNA damage are less resistant to the action of chemical mutagens.

In mammalian cells, DNA repair processes can be grouped into four classes: (i) excision or pre-replication repair; (ii) strand break repair; (iii) post-replication repair and (iv) photoreactivation. The last one has only been induced by ultraviolet rays while the former three are known to be made up of multiple pathways. The biological manifestation of the type and quantity of repair may depend on the state of the cell cycle ($G_1$ or S) and/or the state of differentiation of the tissue.

*Excision repair* involves the removal of damaged DNA by a complex of enzymes. The damaged segment is removed and replaced by error-free DNA synthesised using the opposite strand as template. It includes four general steps: incision, excision, polymerisation and ligation. The first step involves the recognition of the damaged site in the DNA by an endonuclease or N-glycosidase. Various repair endonucleases have been isolated from both mammalian and bacterial cells. Generally, they make strand breaks near DNA lesions. The second step, the excision process, involves the activity of an exonuclease that degrades denatured, but not native DNA. As a result the damaged region is released, together with a limited number of other nucleotides, depending upon the form of damage induced. In the third step, repair replication takes place. Using a DNA-repair polymerase, a new segment is synthesised from the undamaged opposite strand of DNA as a template. In the final step, the newly synthesised DNA is sealed to the parental DNA by a polynucleotide ligase. A mutation may occur during this step if the nicked DNA is released by the ligase before the damaged segment has been removed. In an alternative method, the base is split from the deoxyribose, catalysed by a glycosylase, leaving an apurinic or apyrimidinic site (AP). In the next stage, two pathways are possible. In one, an AP-specific endonuclease nicks the DNA, removing the deoxyribose lacking a base and a few adjacent nucleotides, making it a *short patch repair*. The later steps are possibly similar to nucleotide excision repair. In the second pathway, on the other hand, an undamaged base is simply added to the vacant site of the damaged base.

Chemical agents form adducts in which the agent is bound to DNA by covalent bonds, although some chemicals may break the DNA thread directly. The kinetics of repair varies for the adduct sites as well as for the cell types. Base excision repairs may act in the repair of many simple adducts, like the alkyl groups. Nucleotide excision repair may be responsible for the repair of bulky addition products such as those formed by the carcinogen aflatoxin B.

The various forms of prereplication or excision repair show certain similarities in the mechanism involved though these are controlled by several other factors, including the pattern of chromatin association with DNA.

*Strand break repair*: Breaks in the DNA strand may be induced by a number of agents, though single strand break *in vivo* appears to be mediated by the hydroxy radical. When such breaks appear adjacent to one another on opposite DNA strands, a double strand break is formed.

*Post-replication repair* involves the repair of newly synthesised DNA from a defective or damaged template. It is important only in cells actively synthesising DNA and cells that were unable to excise all lesions from their DNA before the onset of DNA synthesis.

Unrepaired DNA lesions may induce mutations through gene derepression, viral transformation or integration (see chapter on Genetical basis of cancer). Error-prone repairs may also lead to mutations, particularly in cells of the higher organism. Another form of repair may result in genetic recombination, which can be easily distinguished from the other types.

Ionising radiation causes base damage, single and double strand breaks and complex lesions on the DNA. Repair of single strand break and base damage is almost immediate. The damage induced by ultraviolet rays involves the base pairs, mostly in the form of pyrimidine dimers, DNA-DNA and DNA-protein crosslinks. The time period for the repair of such dimers through nucleotide excision may take even a few days.

### C. Modern concept of the gene and origin of cell organelles

(see Sharma AK 1984 In: *Chromosome Structure*, ed Datta SK, CRC Press, USA).

The two groups, prokaryotes and eukaryotes, are only linked by the universality of the genetic code. The former, though traced back to three billion years ago, still maintains a relatively simple genetical structure with a long chain DNA molecule. The eukaryotes, on the other hand, have been recorded only up to one billion years ago. They show a highly complex cellular structure and a very wide diversity of forms, including some relatively simple ones. There is no fossil evidence linking the two groups.

In the eukaryota, the nuclear membrane delimits the chromosomes, protecting them from the environmental fluctuations to which the cytoplasm is subjected. But at the same time the membrane also acts as an intermediary in controlling gene action, by permitting the transfer of gene products like rRNA and mRNA from the nucleus to the cytoplasm through the nuclear pores. Electron microscopic studies have shown the participation of disorganised parts of the membrane during the divisional cycle in the formation of mitochondria and chloroplasts and also the endoplasmic reticulum.

The mitochondria and chloroplasts have self-replicating DNA molecules resembling those of virus and bacteria in their circular nature. Even the ribosomes in chloroplasts and mitochondria are similar to prokaryotes (70S) and are unlike the 80S ribosomes of eukaryotes.

Based on these evidences, a prokaryotic origin has been suggested for these cell organelles. According to the theory of endosymbiosis, in the early stages, unicellular amoeboid *eukaryotes*, unable to photosynthesise, engulfed bacteria able to utilise solar energy for photosynthesis. These two mutually dependent forms gave rise to the modern day chloroplasts as shown by the similarity between the photosynthetic lamellae of the chloroplast and photosynthetic bacteria.

A similar combination of an amoeboid form with a *non-photosynthetic* bacterium may have led to the formation of mitochondria in the later stages of evolution. The resemblance of ribosomes to prokaryotes in structure may also suggest a similar origin. The process has obviously been accompanied by a loss in autonomy, but not to the same extent, of the participants of the symbiosis.

Mitochondria are largely, and their envelopes entirely, dependent on nuclear genes. Most mitochondrial proteins are also controlled by nuclear genes, as also the recombination between mitochondria of two species. The relatively few (about 30) genes mapped on mitochondrial DNA are unable to account for the wide spectrum of proteins required for the complex functioning of mitochondria, which are doubtless controlled by nuclear genes as well.

On the other hand, chloroplasts have retained much greater autonomy. About 300 genes have been mapped on chloroplast DNA and though nuclear genes are involved, their control is less than in mitochondria. In some cases, a single chloroplast gene and two nuclear genes may exert a dual control in the formation of a particular chloroplast protein.

An alternative theory to endosymbiosis is that eukaryotes have originated from a well-organised non-photosynthetic prokaryote with respiratory genes. However, it has not received much support. Later workers have suggested that prokaryotes and eukaryotes had a common progenitor but the evolution of the two groups has been parallel rather than one being derived from the other.

The DNA of a eukaryotic chromosome has structural operator and repressor genes. The mRNA is produced during transcription from an operon with the aid of DNA-dependent RNA polymerase and it moves on to the ribosomes to produce proteins through translation. It was observed that when the mRNA is first produced, it equals the parent gene in length but when it reaches the ribosomes, the length is found to be shorter. The reduction in length of the primary transcript is known as the *processing of the messenger*. Immediately after the formation of mRNA through transcription, a long chain *poly A* is attached as a tail to one end, to guide the mRNA to the ribosomes. The presence of methylated sequences at the end of mRNA protects it from forming other attachments, a process known as capping. Since the ultimate mRNA molecule is shorter than the primary transcript, obviously certain segments are absent from it. This lack however does not affect the code or the subsequent protein produced and therefore the gene containing the code must have been *split* with certain segments not required.

In the DNA molecule, intervening sequences of nucleotides are often observed between structural genes. These are referred to as *introns* while the necessary genes which are separated by the introns are called *exons*. The *split gene* concept of eukaryote thus visualises genes to be split in nature with intervening sequences in between exons. The introns may be non-functional depending on the requirements of transcription. During transcription, the entire gene, including introns, is transcribed to form a mRNA of the same length. Restriction endonucleases, specific for particular purposes, excise the requisite number of nucleotides, thus *splicing off* the introns. These are then joined together by polynucleotide ligase so that the ultimate translatable mRNA is much shorter and poly A is then attached to it.

Since the whole set, split genes, introns and shortened transcripts, is not observed in the prokaryotes, the concept of the prokaryotic origin of the eukaryotes was questioned and the idea of parallel evolution from a common progenitor was put forward.

This modern concept of gene can be reconciled with the endosymbiotic theory of the origin of cell organelles by considering that one partner of the endosymbiosis was a primitive eukaryote with the property of splicing and split gene. It engulfed or entered into symbiotic relationship with two prokaryotes, one with the property of photosynthesis and the other with that of respiration to give rise to ultimately a eukaryotic cell with nucleus, chloroplast and mitochondria (Sharma AK 1984) [illegible] genes, [illegible] other attributes [illegible] [illegible] the [illegible] [illegible] of [illegible] [illegible] [illegible] [illegible] [illegible]

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## SECTION IV

# CHROMOSOMAL ALTERATIONS

## CHAPTER IV: 1

# INTRODUCTION

**TYPES OF CHANGES**: The chromosome complement, usually referred to as karyotype of a particular taxon, is regarded as constant in number and structure for that particular taxon. The two parental sets or *genomes* are generally identical so that each chromosome can pair with a homologue during meiosis. However, this basic constancy can be altered, either spontaneously or through artificial means, giving rise to variations, which may or may not survive, depending on the amount of alteration involved and the environmental selection pressures.

Such changes or variations can be: (i) change in the structure of the chromosome; (ii) change in the number of chromosomes within a complement; and (iii) change in the entire complement.

Structural changes in the chromosomes involve: (a) submicroscopic changes at the molecular levels, that is, point mutation or gene mutation; and (b) alterations observable at the level of the light microscope, including gross changes of chromosome segments.

The difference between these two types is obviously one of degree, depending on the size of the segment and the degree of resolution of the microscope. With progressive sophistication of technology, the boundaries between the two may merge.

Change in the number of chromosomes within a complement may involve the addition or loss of one or more individual chromosomes. It may or may not be accompanied by structural rearrangements. Similarly a change in the entire complement may involve multiplication of the whole haploid set, leading to different levels of polyploidy, with or without structural alterations.

**Variations in complement**: The chromosome complement of a species, though usually constant, shows certain variations, involving the structure or number or both. They include:

(i) variation in chromosome complement between two sexes;

(ii) variation in complement between cells of germ line and soma;

(iii) variation in chromosome complement between individuals of a population, due to balanced genetic polymorphism;

(iv) variation within a species, due to presence of chromosomal races or cytotypes; and

(v) variation between the cells of a single individual; either all the cells would carry a complement differing from the other members of the population; or two or more types of complements may be present in the different cell lines, forming a mosaic.

## CHAPTER IV: 2

# ALTERATIONS IN CHROMOSOME STRUCTURE

When both the genomes of an individual are identical, without any structural changes inherited from either parent, the individual is a *homozygote*. If however, a structural change has occurred in one chromosome but not in its homologue, the complement is *heterozygous* for the change. Alternatively, both homologous chromosomes may carry the same alteration, making the complement *homozygous* for the alteration. Homozygotes form bivalents during meiosis while heterozygotes, depending on the nature of the change, exhibit different types of chromosomal behaviour. Heterozygotes involving minor alterations or alterations of heterochromatic segments have a greater chance of survival since there will be less gross alterations in the phenotype.

The alteration of a chromosome segment, may, according to its size, affect several genes, and therefore, several sets of phenotypic characters. Such changes in chromosome structure are caused by two events, breakage and reunion, according to the classical theory. All breaks are essentially similar and fracture possibly the same types of chemical bonds.

**TELOMERE THEORY:** The telomere theory of HJ Müller, based on the presumption that terminal changes due to a single break alone are not possible, regards the natural chromosome ends—or telomeres—as different from freshly broken ends. Telomeres are "non-sticky" and therefore not capable of fusing with other telomeres or with freshly-broken "sticky" ends. As a corollary, chromosomes without telomeres are unstable and are lost during cell division. The application of the banding pattern techniques has shown however that in many cases at least, this hypothesis is not tenable.

HYPOTHESIS FOR REARRANGEMENTS: Of the two hypotheses regarding chromosomal rearrangements, the earlier classical theory of Stadler, Sax and Müller suggests the occurrence of two separate events—breakage followed by rejoining of the broken ends in a different manner. When a large number of breaks is produced, as for example by irradiation, most undergo "restitution" with their original partners and are indistinguishable in the subsequent metaphase. An *exchange hypothesis*, on the other hand, postulates that rearrangements are primarily at the chromatid level and are caused at the regions where two strands cross each other. If the two strands belong to the same chromatid, an *intrachange* takes place and if they belong to different chromatids, an *exchange* will occur. Both may be complete, with two rejoinings or incomplete, with one rejoining and two broken ends remaining unjoined. Four types of interchanges can be visualised, all of the complete type or belonging to one of the two alternative incomplete types. The resultant chromosomal alterations will be: (i) a small direct tandem duplication in one chromatid and a deletion in the other; (ii) a short deletion in one chromatid; (iii) a small inversion in one chromatid; and (iv) isochromatid breaks. Exchanges *between* chromatids would also give similar combinations. Since such exchanges or translocations are non-random in nature, the orientation of the chromosomes during interphase may determine the frequency of the different types of translocations. These two hypotheses of chromosomal rearrangement may, however, not be mutually exclusive. In maize, chromosomal alterations have been shown to arise through fusion of chromosome ends broken mechanically by stretching across the spindle, due to the occurrence of breakage prior to reunion. Evidences of chromosomal changes induced by physical agents appear on the other hand to be more in favour of the exchange hypothesis. Both random and non-random rearrangements are observed. It is possible that breaks followed by rejoining are responsible for the random changes while exchanges account for the non-random ones.

When visualised in terms of the DNA molecule, in all chromosomal alterations, rejoining must maintain the polarity of the two strands of the double helix.

DIFFERENT TYPES OF ALTERATIONS: The different types of structural aberrations are: deletion and deficiency, duplication, inversion and translocation. The first two types are *quantitative* changes, involving loss or gain in chromosome segments while the latter ones are *qualitative*, where only structural rearrangements take place, the total amount of chromatin matter or genes remaining the same. The fate of the chromosome bearing the alteration depends on the position of the centromere and the amount of heterochromatin present.

### Deficiency and deletion (Fig. IV: 2.1)

When a chromosome loses a segment at one end, the phenomenon is known as deficiency and the chromosome is regarded as a *deficient* one. Loss of an intercalary segment is known as *deletion*. It takes place by breakage across the coiled region of a chromosome and rejoining of the broken ends to form two segments. In the original chromosome *abcde*.a loop is formed. At the point of contact between the chromosome strands, a break and a new union occur, leading to two new parts—a rodshaped chromosome *abe* with a deletion and a ring shaped part *cd*. Deletions are much more frequent than deficiency. *Amphiplasty* or the loss of a terminal satellite by breakage across the satellite stalk and attenuation of the chromosome is a form of deficiency observed in certain plants, which does not fit in with the telomere hypothesis.

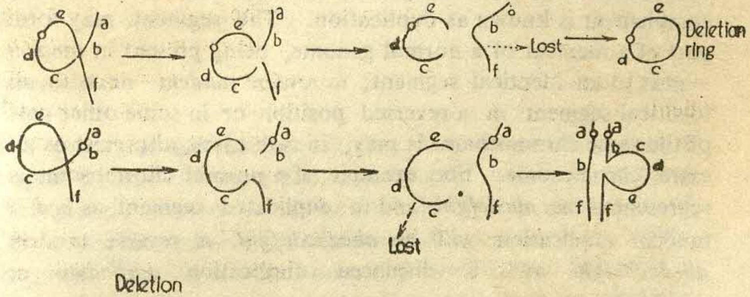

Fig. IV: 2.1

The *fate* of a deficient chromosome depends on the position of the centromere and the size of the portion deleted. The segment with the centromere, if it contains most of the major genes, survives but the fragment without the centromere (*acentric* fragment) is lost. When the centromere is located within the coil and the acentric fragment is a small one, the broken ends of the coil may rejoin to form a *ring* chromosome. If the deleted segment is large, with a number of genes, even the fragment with the centromere may be lost, since the gamete bearing such a chromosome depleted of several genes, will be sterile. In the case of plants and animals with diffuse centromere (holocentric chromosome), like *Luzula* and some members of Homoptera, both fragments appear to survive in succeeding generations.

*Meiotic* behaviour of chromosomes with deficiencies shows that the deficient chromosome pairs with a normal one along their homologous portions, the portion corresponding to the broken segment remaining unpaired. A deleted chromosome also pairs with a normal one along the homologous parts and the extra segment of the normal chromosome bulges out as a loop.

*Survival* depends upon the size of the segment lost. Individuals heterozygous for a small deletion or deficiency may survive since the other homologue carries the full complement of genes. Homozygotes for deficiency and deletion have very little chance of survival.

### Duplication (Fig. IV : 2.2)

The presence of an extra segment of a chromosome of a normal complement is known as duplication. The segment may form part of a member of a normal genome, being present in *tandem* —next to an identical segment; in *reverse tandem*—next to an identical segment in a reversed position or in some other part of the same chromosome. It may, in rare cases, also exist as an extra chromosome. For example, if a normal chromosome is represented as *abcdefghi* and a duplicated segment as *bcd*, a tandem duplication will be *abcdbcdefghi*, a reverse tandem *abcddcbefghi* and a displaced duplication *pqrbcdstu* or *pqrdcbstu*.

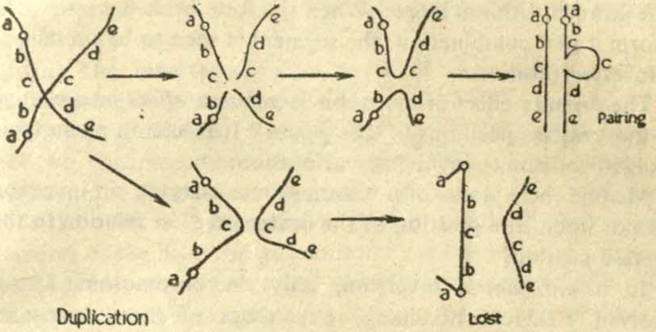

Fig. IV : 2.2

Duplications may arise as follows: two homologous chromosomes lie across each other and at the points of contact, two breaks occur, followed by possible recombinations of the four chromosome ends. The result is two new chromosomes, one of which (*abe*) represents a deletion and the other (*abcdcde*) a duplication.

Duplications are observed more frequently in nature in the heterozygous condition and are less likely to be lethal to an individual than deficiencies. During meiosis, while pairing with a normal homologous chromosome, the duplicated segment may bulge out as a loop. Homozygotes, however, have little chance for survival unless the segment involved is very small.

A well-known example is the *Bar* phenotype of *Drosophila* associated with tandem duplication. It involves 4 or 5 bands in the 16A region of the salivary gland chromosomes. In normal males the 16A region is present once, in *Bar* males twice and in *Bar-double* males thrice.

## Inversion

The structural alteration occurring most frequently in higher organisms in wild state and utilised most widely in genetical experiments, is *inversion*. It involves the reversal in position of a chromosome segment. It is almost always intercalary, rarely terminal. The formation of inversion involves two breaks in a single chromosome at the point of contact between chromosome strands within a loop. When the four broken ends rejoin to form a new combination, the segment is seen to be present in an inverted position.

The primary effect of inversion is *position effect* or a change in the relative positions of the genes. It results in phenotypic changes, sometimes involving variegation.

Meiotic behaviour of a chromosome carrying an inversion depends upon the position of the centromere in relation to the inverted portion.

In a *paracentric* inversion, only one chromosome arm is involved. There is no change in the shape of the chromosome concerned. This type is more frequent than the other. A *pericentric* inversion includes the segment bearing the centromere. It may be *symmetrical* in which the centromere is located close to the middle of the inverted segment, or *asymmetrical*, in which the centromere is not in the middle. The latter type results in a change in the chromosome morphology and can be detected easily.

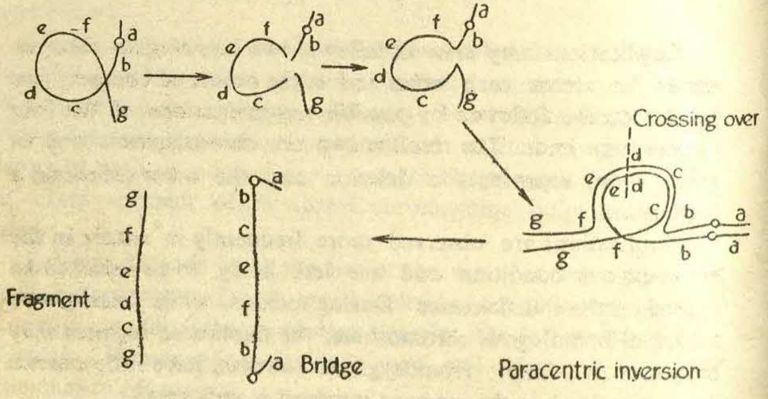

Fig. IV: 2.3

The possible configurations during meiosis include:

(i) Both the chromosome with the inversion and its normal homologue do not pair but remain as univalents giving rise to fertile gametes.

(ii) The two homologues pair along their homologous segments, the inverted part and the region homologous to it remaining as two opposite loops. Disjunction will result in fertile gametes, with 50% bearing the chromosome with the inversion and 50% the normal one.

(iii) The inverted segment may loop over itself to pair with its homologous counterpart in the normal chromosome. In a paracentric inversion, a crossing-over may take place between the two chromosomes within the loop. When the chromosomes separate, one chromosome will have two centromeres (dicentric) while the other will be acentric. During anaphase, the two centromeres of the dicentric chromosome tend to move to opposite poles, giving rise to a typical dicentric inversion bridge. The acentric chromosome remains at the equator or may divide and two acentric fragments may be observed at the two poles. The bridge may break in the middle, the two broken pieces moving to different poles or the entire bridge may be lost during cell division. In rare cases, as for example in *Triticum* reported by Sears, the entire bridge may move to one pole and be maintained as such through successive cell divisions. However, in all cases, after the formation of the inversion bridge, both the bridge and the fragment are ultimately eliminated. If included within a gamete, it is sterile. Double fragments and double bridges may arise if all the four chromatids are involved in the crossing-over. If the bridge remains at the equator, 50% of the gametes are non-viable, and if included within one daughter cell, that gamete becomes non-viable (Fig. IV : 2.3).

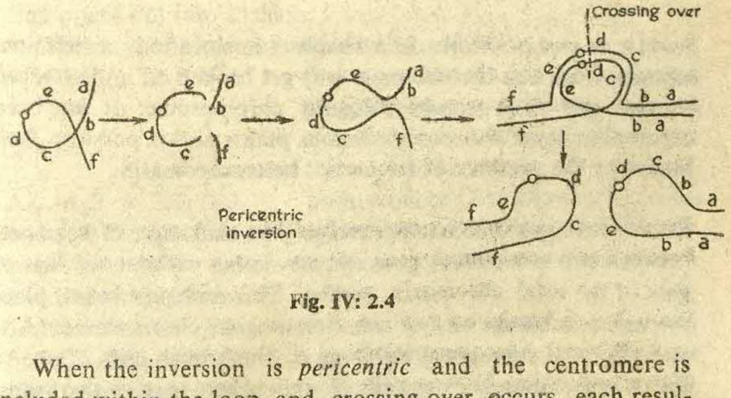

Fig. IV: 2.4

When the inversion is *pericentric* and the centromere is included within the loop, and crossing-over occurs, each resultant chromosome has a centromere. However the compositions of both the daughter chromosomes have been altered and therefore inversion bridge is not formed. In the changed segment, some genes are duplicated and others are deleted. The phenotypic effects are accordingly altered (Fig. IV: 2.4).

Inversions can be recognised in man easily if the arm ratio is altered, i.e., if they are asymmetrical pericentric. They have been observed in the larger chromosomes and have been associated with reduced fertility. A study of the somatic banding patterns can also be utilised to locate small inverted segments.

### Translocations

Involve interchromosomal or intrachromosomal rearrangement by the exchange or addition of segments. Several types of translocations have been recorded, depending on the number of breaks and rejoining: (i) simple translocation; (ii) reciprocal translocation; (iii) shift within the same chromosome; (iv) insertion to a different chromosome, between non-homologous or homologous chromosomes; and (v) centric fusion.

Type (i) involves a single break, type (ii) two breaks and types (iii) and (iv) three breaks. All these types have been observed in man leading to the formation of congenitally abnormal propositii. They sometimes result in lethality and subsequent abortion, at others in infertility or congenital abnormalities though in most cases, they have not been correlated with definite syndromes.

SIMPLE TRANSLOCATION: In a simple translocation, a terminal segment from one chromosome may get broken off and attached to one end of a non-homologous chromosome. It has been reported in some monocotyledonous plants and is possibly facilitated by the presence of telomeric heterochromatin.

RECIPROCAL TRANSLOCATION involves the exchange of segments between two non-homologous chromosomes without any loss or gain of the total chromatin matter. This exchange takes place through two breaks on two non-homologous chromosomes ($AA_1$ and $BB_1$) and subsequent rejoining of the broken ends of one to the corresponding broken ends of the other to give two translocated chromosomes $AB_1$ and $BA_1$ (Fig. IV: 2.5).

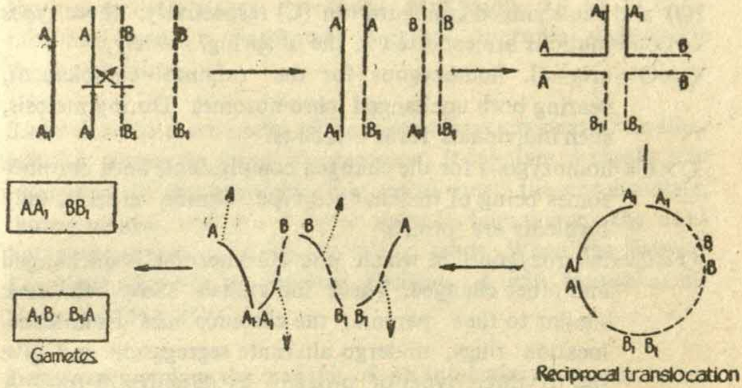

Fig. IV: 2.5

Two types of such mutual translocations are possible: In one, two new monocentric chromosomes are formed (symmetrical interchange) while in the other, a dicentric and an acentric chromosomes arise (asymmetrical interchange), which are not transmissible. In cases of symmetrical interchange, when the two translocated chromosomes ($AB_1$ and $BA_1$) undergo pairing with their normal homologues ($AA_1$ and $BB_1$) during meiosis, the four chromosomes pair along their homologous segments. A cross is formed at first, which is converted on the terminalisation of the chiasma to give a translocation ring. Later the ring opens out into a chain.

Three types of combinations are possible:

| | |
|---|---|
| $AA_1\ BB_1 \times A_1B\ B_1A$ | that is, a normal and a translocated chromosomes, produced by alternate chromosomes of the chain going to the same pole. |
| $AA_1\ A_1B \times BB_1\ B_1A$<br>and $A_1B\ BB_1 \times B_1A\ AA_1$ | both produced by adjacent chromosomes going to the same pole. |

In the two latter cases, duplications and deletions are present and the combinations are lethal.

Therefore the only possible viable configuration is the first one $AA_1\ BB_1$ and $A_1B\ B_1A$, in which alternate chromosomes have moved to the same pole. Thus, of the two sets of viable gametes formed, one bears the original chromosome $AA_1\ BB_1$ and the other the translocated one $AB_1\ BA_1$.

From these two gametes, with the original configuration (O) and the changed configuration (C) respectively, three types of combinations are expected in the offspring, namely,

O×O=original, homozygous for the original complement, bearing both unchanged chromosomes. During meiosis, such individuals form bivalents.

C×C=homozygous for the changed complement, both chromosomes being of the changed type. During meiosis, only bivalents are formed.

O×C=heterozygous, in which one chromosome is unchanged and other changed. These individuals show behaviour similar to their parents; the chromosomes form translocation rings, undergo alternate segregation and give rise to three types of progeny as described before. A translocation involving three chromosomes will give a ring of 6, of 4 chromosomes a ring of 8 and so on.

In *Datura stramonium*, all the three types of progeny, O×O, O×C and C×C have been observed.

In certain genera, like *Oenothera* (n=7), one species *O. hookeri* has 7 bivalents and the chromosomes are unchanged (O×O). Other species, like *O. biennis*, show the progressive formation of rings of 4, 6, 8 and 10 chromosomes, giving a series of C×O configurations where 2, 3, 4 and 5 chromosomes have undergone reciprocal translocations. Finally, in *O. lamarckiana*, a ring of 14 chromosomes is observed, suggesting that all 7 chromosomes have entered into reciprocal translocations with each other. The ring of 14 later opens out in a chain. Adjacent chromosomes go to opposite poles during anaphase, forming two sets of 7 chromosomes, named gaudens and velans —which remain constant through successive generations. The entire complex is named the Renner complex after its author. This type of translocation heterozygote, in which only the combination C×O is possible is known as a balanced heterozygote. Any other combination, homozygous for either gaudens (G×G) or velans (V×V) is not viable either due to *heterogamy* —the relatively better sponsoring of the velans complement in the ovule and the gaudens in the pollen mother cell—or due to a gene causing *balanced lethality*, which is lethal in deficient or duplicate doses.

The monotypic genus *Rhoeo discolor* of the family Commelinaceae is an ultimate type of balanced heterozygote, in which the intermediate stages have been eliminated. All its 12 chromosomes form a ring and undergo alternate segregation during anaphase.

SHIFT denotes the transfer of an intercalary segment to another position within the same chromosome. It involves 3 breaks and rejoinings. If the segment is a small one, the chromosome, during meiosis, will pair with its normal homologue, the non-homologous portions bulging out as loops. When the segment has been placed in an inverted position, it will behave as an inversion (Fig. IV: 2.6).

INSERTION involves the transfer of an intercalary segment to an intercalary position in another chromosome, which may be homologous or non-homologous with the original chromosome.

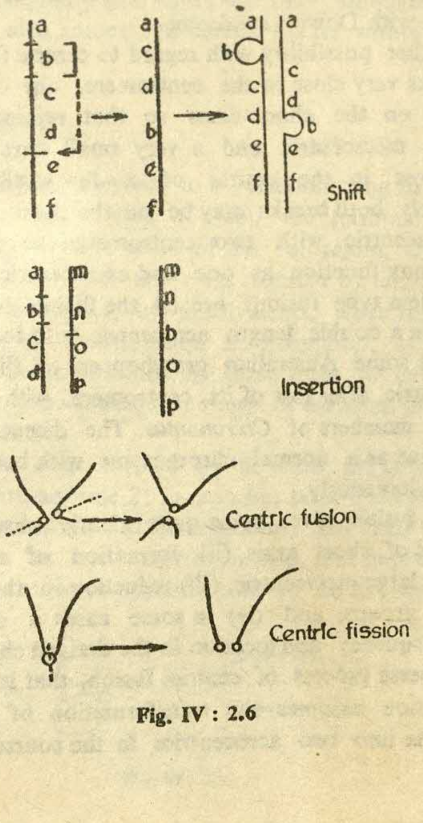

**Fig. IV : 2.6**

If inserted within a homologous chromosome, the original one will be deficient and the one with the insertion will bear a duplication for the segment concerned. Meiotic behaviour of the two chromosomes will be modified accordingly. When the insertion is in a non-homologous chromosome, the behaviour will depend upon the size of the segment. The banding pattern methods have provided another tool to identify insertions.

CENTRIC FUSION OR ROBERTSONIAN TRANSLOCATION involves reciprocal translocation across the centromeric regions of acrocentric chromosomes or the fusion of telocentric chromosomes, to form a metacentric chromosome. It is a special type, not strictly involving breakage, and has been observed principally in mammalian chromosomes. In man, centric fusion has been observed between two acrocentric chromosomes, involving a No. 15 and a No. 21 or both No. 21, to give a metacentric associated with Down's syndrome.

The other possibility with regard to centric fusion includes both breaks very close to the centromere, one on the long and the other on the short arms so that rejoining gives rise to a large metacentric and a very small chromosomes. The latter is lost in the course of a few cell generations. Alternatively both breaks may be on the short arms, leading to a metacentric with two centromeres so close together that they may function as one and an acentric chromosomes. Other tandem type fusions are: (i) the fusion of two acrocentrics to give a double length acrocentric with loss of a centromere, as in some Australian grasshoppers or, (ii) the fusion of an acrocentric, after loss of its centromere, with a metacentric, as in some members of *Chironomus*. The dicentric may sometimes behave as a normal chromosome with both centromeres active simultaneously.

Centric fusion gives some quite clearly observable results, like (i) loss of short arms, (ii) formation of a new linkage group: the large metacentric, (iii) reduction in the total number of linkage groups, and (iv) in some cases a modification of chiasma frequency and location in the derived chromosome.

The reverse process of centric fusion, that is **centric fission** or dissociation assumes the transformation of a metacentric chromosome into two acrocentrics in the course of evolution.

It is regarded by some workers to occur by simple fission through the centromere. The hypothesis postulated by White (MJD 1973 *Animal cytology and evolution* 3rd ed Cambridge University Press London) however assumes that this process involves a gain of a centromere and two telomeres, by translocation with a minute centric donor chromosome.

According to the telomere concept, both centric fusion and dissociation are special types of mutual translocations involving the loss and the gain respectively of one centromere and two telomeres. These two processes can increase or reduce the chromosome number without a change in the number of long arms, according to Robertson's law that the number of chromosomes may vary but the number of arms remains constant. They have been found to be responsible for polymorphism in different species, as for example, in the rainbow trout *Salmo iridens*, where polymorphism exists not only amongst different individuals but also amongst different tissues within the same individual.

## Ring chromosomes

The term is usually applied to a deletion ring in which the centromere is in the middle of a coil, the terminal segments break off and the broken ends of the coil rejoin. Other rings observed during meiosis are rings formed by large bivalents opening out at diakinesis; by multivalents, when the chiasma are terminalised; by translocation heterozygotes, as described earlier and by isochromosomes. All these rings can be distinguished on observing the subsequent stages of division, particularly anaphasic segregation.

Some centric rings formed by deletion are stable, as for example, ring chromosome 21 in man has been observed to give an anti-mongoloid slant. More commonly they tend to change in size or even be lost. During mitosis, the duplicated ring chromatids may move to opposite poles to be included in dau-

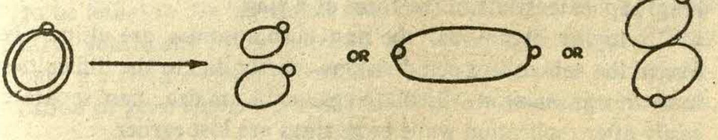

Fig. IV: 2.7

ghter cells; they may form a double-sized ring or an interlocking ring (Fig. IV: 2.7).

Further division results in: (a) the interlocking ring may break, segregate regularly and fuse; (b) a dicentric ring may break asymmetrically and the resultant chromosomes will carry the duplication-deficiency complements; (c) the ring may be lost by anaphase lagging; or (d) the ring may be included in either pole through non-disjunction.

The subsequent cell generations may carry complements varying between monosomy and tripolysomy, with variable duplication-deficiency phenotypes (Fig. IV : 2.8).

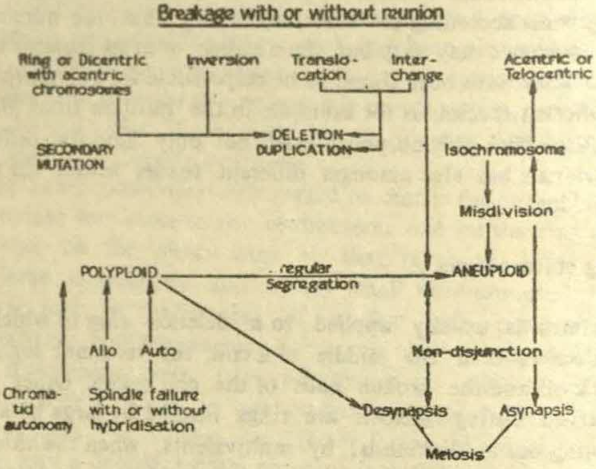

**Fig. IV:2.8 Chromosome mutations and their relationship (after Lewis and John 1963 *l.c.*).**

Examples of ring chromosomes have been observed in patients with variable malformations including 18r and 21r. In lower groups like viruses, the ring chromosome may occur naturally. The lambda bacteriophage chromosome is often in the form of a circular ring due to the complementary redundancy of the single polynucleotide sequences which are present at its terminal regions. The chromosome of *Escherichia coli* (1 mm long) replicates while in the form of a ring.

In higher organisms, the ring chromosomes are ultimately lost in the subsequent cell divisions, partly due to the difficulties in their transmission. Small rings, as in maize, can separate easily after replication while large rings are lost earlier.

## CHAPTER IV: 3

# RELATIVE ROLES OF CHROMOSOMAL CHANGES IN EVOLUTION

### Deficiency

Since deficiencies result in gene loss, they may give rise to recognizable genetic consequences, which again may act as recessive lethals, as for example the dominant characters *blonde*, *pale*, *beaded*, *carved* and *shipped* in *Drosophila* are all associated with deficiencies. In some cases, there is even a clear correlation between the phenotypic effect and the number of bands of the salivary gland chromosome deleted. Similarly, in maize viable morphological variants can be produced by homozygous tiny deficiencies. However, deficiencies do not appear to have a concrete effect on evolution since they involve diminution in metabolic control. Reduction in chromosome number and/or chromosome size, as observed in *Crepis*, involves reciprocal translocation and the loss of the centromere and its associated heterochromatin. Since heterochromatin represents repressed regions of the genome, such losses may not be detrimental. However, the application of the banding techniques to such cases where a loss of chromosome segment appears to accompany specialisation may further clarify the nature of the segment involved.

### Duplications

Duplications occur more frequently in nature and are less likely to be lethal to the individual than deletions. They can be recognized genetically by the absence of recessive phenotypes. They have a certain amount of evolutionary significance since duplication of gene loci is a feasible method for acquiring new genes, resulting in new physiological functions. Thus two identical genes, one arising from the other through duplication, gradually may diverge through a series of mutations. At a later evolutionary stage, they may ultimately control completely different functions. Such duplications, most frequently tandem repeats, have been established in the salivary gland chromosomes of *Drosophila*. Duplication at the genic level has also been proved to be the major factor in the evolution of human haemoglobin. The most common form of haemoglobin, haemoglobin A (Hb A), contains four polypeptide chains, two alpha and two beta. In a variant form Hb $A_2$, two delta chains are present instead of the two beta. The loci for beta and delta are closely linked and have possibly arisen, one from the other, through duplication. In an abnormal variant termed Hb Lepore, two of the loci have the usual alpha chains while the polypeptides of the other two have amino-acid sequences characteristic of both beta and delta chains. Unequal crossing-over between the beta and delta loci may have given rise to a deleted segment in one chromosome and a duplicated one in the other. The deleted chromosome would have parts of the beta and delta loci close to each other. The whole segment would be activated when producing haemoglobin rather than the two loci independently. By transcription and translation, the Hb Lepore variant would be synthesised from this segment.

The possible role of chromosome duplications in the evolution of higher mammals has been discussed later in this chapter.

### Inversions

Inversions of the paracentric type have been studied extensively for their evolutionary significance. They have been recorded in a large number of organisms, from plants like *Paris* to many species of *Drosophila*. They have not been observed in certain groups, including *Anopheles* amongst the mosquitoes, the urodele amongst the amphibians and many grasshoppers. The reassessment of these genera with the aid of banding techniques may however reveal the small inversions which may be undetectable cytologically.

An instance of sequential inversions giving rise to new forms is provided by the third chromosome of *Drosophila pseudo-*

*obscura* shown by Dobzhansky. In this species and its related *D. persimilis* a number of inversions can be shown to be related to each other, as in the accompanying figure (Fig. IV: 3.1). These inversions show a certain pattern of correlation with the region and the season of their occurrence. Thus inversions may be actually selected for in natural populations. The segments involved, in the absence of crossing-over, may become allelically different from similar segments due to the accumulation of random mutations in these regions. Selection can operate more successfully on such partial gene sequences instead of on single genes. If the inverted segment is too long, it may be involved in recombination. It must be of a sufficient length however so that it can collect a sufficient number of gene changes which would permit it to respond differentially to environmental changes. In *Drosophila* such inversions have a definite selective value. The accumulation of mutations at these segments may in time give rise to sterility barriers from the parental type, leading to the formation of new species.

Considered at the level of the DNA molecule, in an inverted segment, the gene order is inverted and the polynucleotide segments must change positions. During transcription, the mRNA

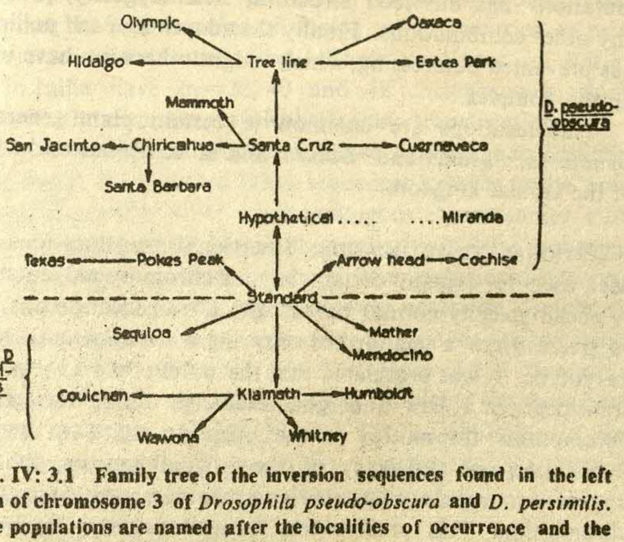

**Fig. IV: 3.1 Family tree of the inversion sequences found in the left arm of chromosome 3 of *Drosophila pseudo-obscura* and *D. persimilis*. The populations are named after the localities of occurrence and the relationships have been established through overlapping inversions (Dobzhansky).**

formed by one of the polynucleotides must be correspondingly altered, giving altered protein synthesis. Some inversions do not however give any phenotypic effect.

### Translocations

RECIPROCAL PROGRESSIVE TRANSLOCATION: In reciprocal progressive translocation *heterozygotes*, like *Datura* and *Oenothera*, the number of independent linkage groups decreases with the increase in size of the translocation complex. Thus the variability due to independent assortment of chromosomes is reduced. The final form like *Oe. lamarckiana* with a ring of 14, has been derived through a series of successive translocations from the original species with 7 bivalents as given in pages 165 and 166. A study of the North American oenotheras shows that they have utilised three genetic devices, each deleterious to a certain extent, in combination to give their present evolutionary diversity. Reciprocal translocation has led to the formation of the diverse linkage groups (the complexes), which have drastically reduced genetic recombination. An accumulation of lethal mutations has enforced structural heterozygosity, preventing any other combinations. Finally the adoption of self pollination has prevented outcrossing, which might otherwise have broken up the complex.

Translocations are common in certain plant genera, like *Oenothera*, *Paeonia* and *Datura*, and in scorpions and roaches of the animal kingdom.

INSERTIONAL TRANSLOCATION: Insertional translocations have been used to explain certain human chromosomal anomalies. A phenotypically normal parent with a translocation was found to give birth to a malformed offspring with apparently similar karyotype. It was postulated that the parent had an insertional translocation. It had undergone exchange during meiosis. The chromosome transmitted to the offspring therefore had both duplication and deficiency of the original genome, though it appeared morphologically similar to the corresponding parental chromosome. This process was termed *aneusomy by recombination* (aneusomic de recombinaison). During meiosis, such changed chromosomes stand a risk of more than 50% of recombination within the region inserted (Lejeune J and Berger R 1965 *Ann Genet* **8**: 21).

ROBERTSONIAN TRANSLOCATION: The Robertsonian translocation is of considerable importance in studying karyotype evolution in mammals. For example, the karyotype of *Mus musculus*, the house mouse (2n=40), has all acrocentric chromosomes while that of the closely related tobacco mouse, *M. poschiavinus* (2n=26) has 14 metacentrics. *The fundamental number* (*NF*) or the total number of long arms in the autosomes is the same in both of them, being 40. Apparently, therefore, evolution in these two species has taken place through a fusion of two acrocentrics to give a metacentric (centric fusion) or a fission of a metacentric to produce two acrocentrics (centric fission). Either process must have been repeated several times. In the Swiss Alps, a number of *M. musculus* individuals were recorded which had varying numbers of metacentric chromosomes corresponding to a decrease in the number of acrocentrics. However, as the number for this species collected from all over the world is usually 40, apparently the Swiss variety cannot be the ancestral one. Therefore, within this genus, obviously evolution has taken place through a series of fusion of acrocentrics to produce metacentric chromosomes. Three cytotypes of *Rattus rattus* in India have 2n=38, 40 and 42 chromosomes, which have evidently arisen through Robertsonian translocation.

These consecutive translocations between different acrocentrics may be due to the repeated DNA sequences present in the centromeres. Centromeres of nonhomologous chromosomes may synapse during meiosis due to the similarity in base sequences of repetitive DNA present in them. Thus occasional crossing over may occur across this region between otherwise completely non-identical acrocentric chromosomes to produce a large metacentric (Arrighi FE 1974 In: *The Cell Nucleus* **2**: 1 Academic New York). Reports of centric fusions in plants are more rare (Jones K 1978 *Bot Rev* **6**: 120). In *Gibasis schiedana* of the family Commelinaceae, Robertsonian translocation has resulted in cytotypes with x=4 from another with x=5.

## Addition of new "heterochromatin"

The application of the banding technique for constitutive heterochromatin (C-banding) has suggested that in certain mammals, species can accumulate heterochromatic material and even form new arms, possibly through saltatory replication of repetitive DNA. In the deer mouse, genus *Peromyscus*, the somatic number is 48, but the NF ranges from 52 to 92. In *P. maniculatus*, many chromosome arms are totally heterochromatic while in *P. eremicus* every chromosome has a totally heterochromatic short arm. In two subspecies of *P. maniculatus* differing in NF, a trend is noticed towards the addition of new heterochromatic arms, thus changing the total karyotype. This trend possibly supplements the other mechanisms for changes in the fundamental number, such as, pericentric inversions and unequal translocations.

## Minute chromosomal rearrangements

Minute chromosomal rearrangements of a type intermediate between single gene mutation and major chromosomal changes observed under the light microscope have possibly been frequent in evolution to a significant extent. The large scale differences observed in DNA values between closely related taxa imply the presence of a large number of duplications and deficiencies of DNA in the complements of evolving taxa. Similarly the observable differences in the amount and distribution of heterochromatic segments in related species indicate minor rearrangements of these segments. The differences in the repetitive DNA amounts of related species also signify changes involving them. The banding patterns serve to emphasise many more such minute rearrangements which were not earlier visible with the conventional techniques. White (1973) has suggested two different evolutionary phenomena involving minute structural altertions: (i) evolution of the heterochromatin and (ii) evolution of individual genetic loci in the euchromatin. The relationship between the relative amounts of DNA, heterochromatin and repetitive DNA in evolving taxa has not yet been fully clarified. Application of the recent methodology to the taxa previously regarded to differ from each other due to cryptic genic changes, like the species of *Quercus*, may show the presence of segmental rearrangements, previously undetected.

CHAPTER IV: 4

# CHROMOSOMAL POLYMORPHISM

## A. Polymorphism for chromosomal alterations

Polymorphism for chromosomal alterations has been recorded in many sexually as well as vegetatively reproducing forms. Genetic polymorphism, at the submicroscopic level, is a universal feature for all populations, since it is the foundation for individual variations. All the different types of chromosomal rearrangements discussed earlier have been recorded in different members of natural populations, including also the presence of accessory chromosomes.

### Chromosomal polymorphism in relation to speciation

Two types of chromosomal polymorphism were recognised by Dobzhansky (T 1951 *Genetics and the origin of species* Columbia New York) based on their positive or negative response to ecological factors. In the *rigid* system, as seen in *Drosophila pavoni*, the frequency of different inversions is constant under varying environmental conditions, while in the *flexible* system, it changes with factors like geographical, altitudinal or monthly variations, as for example, in *D. pseudoobscura.*

FACTORS NEEDED FOR SURVIVAL: The survival of such chromosomal alterations within a population is at least partly due to the fact that the heterozygote for the changed complement is better adapted for existence than both the homozygotes for the changed and unchanged complements under the particular environment to which they have been exposed (*annidation*).

In some cases, the chromosomal alterations are favoured by selection when rare but not when common *(frequency dependent selection)*. Simple heterosis, through which the heterozygote shows increased viability as compared with the homozygotes, is another factor in the production of polymorphism. Alternative types of chromosomes in a population, as for example, those differing by an inversion, are possibly interadapted. In the course of evolution, they may have acquired alleles to interact in the production of viable phenotypes (White MJD 1978 *Modes of Speciation* Freeman San Fransisco).

Those chromosomal rearrangements, however, which are lethal or which reduce the fertility of the heterozygotes to a significant extent, are unable to survive in the heterozygote condition. The tandem fusions, for example, have not been recorded in the natural state. Inversions and translocations, though acting as crossover suppressors over large regions of the chromosomes, are yet often recorded to exist in the polymorphic state. For example, the midge *Simulium vittatum* shows 134 different inversions randomly distributed over the karyotype. Average individuals from different populations are heterozygous for 1.9 to 6.0 inversions, some being more polymorphic than others. Such species escape the consequent infertility attached to a paracentric inversion by *pseudosynapsis* of homologous chromosomes in the germ cells, in which the mutually inverted regions pair nonhomologously straight, without forming a reversed loop. In certain chironomid midges, the percentage of inverted loops is much higher in the polytene chromosomes—which are not involved in reproduction—than in the pachytene ones. In the germ cells of some grasshoppers polymorphic for pericentric inversions, the mutually inverted segments undergo pseudosynapsis in all cases. Pericentric inversions, however, are very much less frequent in populations, because crossing-over results in duplication-deficient chromosomes, which are unable to survive in nature.

The different inversions in a polymorphic population are originally randomly distributed over the karyotype. Later natural selection may eliminate certain combinations, resulting in non-random associations, as observed in various *Chironomus* and *Simulium* species. In the plants, where inversions have been established like *Trillium kamtschaticum* and

*Paris quadrifolia*, the apparently vegetative mode of reproduction has aided in their survival, as discussed in a later section.

Translocation heterozygotes—due to their particular mechanism for maintaining structural heterozygosity—are less frequent in the form of polymorphisms. However, the formation of bivalents in addition to rings has been observed in roaches of the species *Periplaneta americana*. In certain scorpions, belonging to the genera *Tityus* and *Buthus*, both rings and other configurations are seen in meiosis, perhaps due to the holocentric nature of their chromosomes. In the established translocation heterozygote, *Rhoeo discolor*, all possible configurations from bivalents, chains to complete rings of 12 chromosomes can be observed, maintained evidently through its asexual means of propagation.

Polymorphism for chromosomal fusions or dissociations has been recorded frequently, resulting in individual differences in chromosome number in certain groups of animals. During meiotic division in such cases, two acrocentric chromosomes pair with the two arms of a metacentric, forming a trivalent, showing their homology. The mollusc *Thais lapillus* on the Brittany coast has two ecological races. One, with 2n=36, occurs in sheltered areas with limited food supply and another with 2n=26 in exposed areas with unlimited food supply indicating the relative preference of fused and unfused chromosomes for different habitats. The intermediate regions have polymorphic populations with varying chromosome numbers, apparently heterozygotes showing 1 to 5 trivalents in meiosis. The Canadian populations of the ladybird beetle *Chilocorus stigma* show a decreasing chromosome number accompanied by an increasing number of chromosomal fusions of the diphasic type with subsequent loss of heterochromatic arms. They extend across Canada from Nova Scotia to Saskatchewan, most of them being polymorphic for one or more fusions.

Such populations or races, differing in chromosome numbers, have been observed in numerous plant species as well, reproducing partly or wholly through asexual means. However, the mechanisms involved in the changes in number are variable. An outstanding example is the genus *Carex* of the family Cyperaceae, having chromosome numbers ranging from n= 6 to 54, possibly due to the presence of diffuse centromere. Amongst the sexually reproducing plants, reduction in basic number during speciation has been recorded in *Crepis* of the Compositae through reciprocal translocations.

ALLOCYCLY: Polymorphism for allocycly, that is, the presence of uncondensed and therefore, destained chromosome segments, observed after cold treatment, has been recorded in certain plants with large chromosomes. In *Trillium kamtschaticum* (n = 5) in North Japan, the five chromosomes show respectively 7, 14, 15, 16 and 18 different patterns of such decondensed segments varying in their size and distribution in different populations (cf. Stebbins GL Jr 1971 *Chromosome evolution in higher plants* Edward Arnold, London). The distribution of these chromosomal races has been correlated with population size, climate and geological history. It is interpreted as the result of interactions between mutation, genetic recombination, natural selection and genetic drift.

FREQUENCY: The amount of chromosomal polymorphism in nature has been estimated at about 80% in *Drosophila* and *Chironomus* and about 10-20% in grasshoppers and mammals (White MJD 1973 *The Chromosomes* 6th ed Methuen London). This estimate, based on earlier techniques, will have to be considerably modified with the advent of the banding pattern techniques. The banding of polytene chromosomes of the salivary glands formed the ideal material for these studies. If similar identifying bands can be located on mitotic and meiotic chromosomes, polymorphisms for minor structural rearrangements, not visible at present under the light microscope, can be detected. In such an event, the chromosomes of each member of a population would present a series of identifying bands. A collation of the bands would give the spectrum or range of variability within a particular population. The spectrum would differ from that of another population of the same species. The corresponding variations between species and between genera would be in the degree of the range of spectrum exhibited. Karyotypes in such cases would be represented by a spectrum of the variability of the banding patterns manifested by the populations of the taxon.

### B. Chromosomal polymorphism in man

With the advent of the banding techniques, certain heritable polymorphisms are observed amongst the chromosomes of individuals. These cases have mostly been reported in human chromosomes, since these techniques have mostly been developed on them. Such polymorphisms involve chiefly the size, shape or nature of chromosome segments and are minor variations of the structural alterations of the chromosomes described earlier.

Conventional staining methods show chromosome regions which differ to a greater or lesser extent in size, without any apparent phenotypic changes in different individuals, as for example the short arms D and G chromosomes and the long arm of chromosome Y. Q-banding technique has shown that in certain instances these changes may be due to translocation. Intense fluorescence may also be seen in the centromeric region of chromosomes 3 and 4 and on the short arms and satellites of all acrocentrics. These regions are polymorphic. Such fluorescent markers have been located at 5 to 30% sites and the size of the highly fluorescent dots may vary widely in individual chromosomes.

In some cases, a lightly staining segment in the proximal part of the long arm gives a variant chromosome longer than the normal, referred to as the "uncoiler", observed in chromosome Nos. 1 and 9. Clarification of this region with G-banding in chromosome No. 1 shows two dense bands instead of the usual one dense band, suggesting that this variant has arisen through the duplication of a dense band.

Occasionally, a specific region on a chromosome shows an apparent break on both chromatids at metaphase. Such sites are known as "fragile" sites. They occur in a variable number of cells in a spread. The distal segment may replicate at a different rate than the rest of the chromosome. In a low percentage of cells, an extra copy of this segment may be found due to *selective endoreduplication.* The fact that this tendency to break at a particular site is an inherited one led to the assignment of α-haptoglobin to a locus on the long arm of chromosome 16 in man.

### C. Correlation with environment

Amount of genetic variation in a population is an adaptation to the environmental heterogeneity and uncertainty. The latter gives rise to broad niches richer in heterozygosity in contrast to the homozygosity of narrow niches in more stable conditions. Genetic and protein polymorphisms are adaptively important. Environmental heterozygosity probably selects for higher genetic and protein heterozygosity and thereby higher fitness.

An example is the frog. Members of *Pelobatus* sp., the spade foot toads, are subterranean in habit and thereby insulated from short term environmental changes. A study of certain protein polymorphisms based on 26 to 30 gene loci indicates that these animals have significantly low genetic variations. On the other hand, the genetic variation increases progressively with more variable environments, from the edible frogs, *Rana* sp., which are semiaquatic to aquatic to the tree frogs, *Hyla* sp., which are arboreal. The toads, *Bufo* sp., exhibit the highest heterozygosity due to their terrestrial and therefore, most variable habitat.

In general, heterozygosity of enzyme loci may be adaptively neutral or evolutionarily significant, depending on the interaction with environment. Varying and unpredictable environment should select for more heterozygous species while relatively constant environment should select for more homozygous ones. Evidences are however scanty and contradictory.

The evolution of metal resistant plants may involve specific tolerance either one or both of the plant and metal. Such tolerance may prevent uptake of metals through mechanical means. Alternatively it may exclude metal from the metal sensitive sites by the activation of special metal resistant enzymes or alteration of the metabolic pathway. The latter property is obviously gene mediated and may develop within a span of 10-15 years in a population. For example, both *Anthoxanthum odoratum* and *Festuca rubra* are zinc tolerant. In the former, the property has been suggested as due to more than one pair of alleles with partial dominance, selected under conditions of toxicity. Species of *Astragalus* have been used as indicators for metals due to their high affinity for *Se* and *Merceya latifolia* (copper moss) for *Cu*.

Under normal conditions, the heterogeneity involves genes tolerant to metals present in recessive doses but alteration in environment with progressive metal toxicity permits development of plants with dominant tolerance. The populations without latent genetic tolerance to a particular metal, are scheduled for destruction when exposed to such toxicity.

CHAPTER IV: 5

# CHROMOSOMAL ABNORMALITIES IN MAN*

The study of human chromosomes is a discipline of relatively recent activity, encouraged by technical advances during the past two decades. In 1956, the somatic chromosome number in man was correctly reported by Tjio and Levan during the First Congress of Human Genetics at Copenhagen to be $2n=46$ and not 48, as visualised earlier. The record of $n=23$ in the germ cells by Ford and Hamerton in 1959 confirmed this number. In the same year, Lejeune, Gauthier and Turpin associated the presence of an extra chromosome of G type with a specific syndrome and Jacob and Strong showed the role of Y chromosome in sex determination. The deletion of a G type chromosome was correlated with chronic myeloid leukaemia by Hungerford in 1960. These discoveries followed the evolution of a schedule for the study of chromosomes from air dried leucocyte culture, after successive treatments in colchicine and hypotonic solutions, fixation in acetic acid-methyl alcohol and subsequent staining in Giemsa solution. The next decade showed a record spurt of activity. Reports poured in from centres all over the world of aberrations in chromosome number and structure and their probable relationship with congenital malformations. The initial enthusiasm persisted till the mid-sixties. However, since the air-drying procedure remained basically unchanged except for minor modifications, the number of new syndromes discovered started to decrease. The next break-

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*(See also Vogel F and Motulsky G 1979 *Human Genetics* Springer Berlin; Talukder G and Sharma A 1979 *Handbook of Clinical Genelics* Oxford and IBH; Sharma A, Talukder G and Mukherjee SK 1983 *Methods in Human Genetics*, Kalyani Publishers, Ludhiana and National Book Trust).

through came in 1969 and 1970 when, following feeding with the fluorochrome dye, quinacrine mustard, and observation under ultraviolet light, Caspersson, Zech and their colleagues could identify each member of the 23 pairs of chromosomes which constitute a normal human karyotype. The chromosomes were shown to fluoresce in alternate light and dark bands, which, like fingerprints, were constant for each chromosome. In spite of the accuracy of this technique, its technical difficulties prompted research into other procedures more amenable to routine analysis. Banding patterns were obtained after a diversity of treatments, including heating, digestion with acid, alkali or salt, and treatment with a variety of compounds. Out of these numerous protocols, consistent patterns were obtained in four categories: (i) the fluorescent *Q*-bands after treatment with quinacrine mustard, (ii) the *R*-bands occurring in positions reverse to the *Q*-bands observed after controlled heat denaturation, or BUdR pretreatment, (iii) the *G*-bands, produced after treatment in different reagents followed by Giemsa-staining and corresponding to the *Q*-bands in location, and (iv) the *C*-bands, located in regions containing constitutive heterochromatin after denaturation and reassociation (Fig. IV:5.1). The data available at present suggest that the nature of DNA and the availability of its active sites are major factors in banding. Since DNA remains

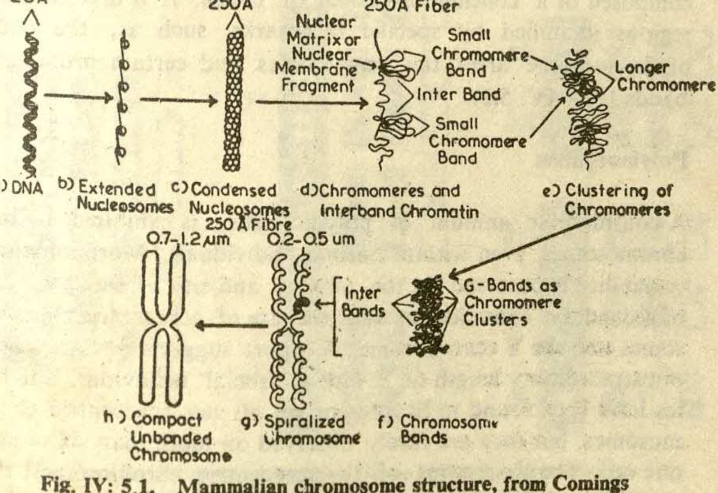
20Å
250Å
250Å Fiber
Nuclear Matrix or Nuclear Membrane Fragment
Small Chromomere Band
Inter Band
Small Chromomere Band
Longer Chromomere
a) DNA
b) Extended Nucleosomes
c) Condensed Nucleosomes 250 Å Fibre
d) Chromomeres and Interband Chromatin
e) Clustering of Chromomeres
0.7–1.2 μm
0.2–0.5 um
Inter Bands
G-Bands as Chromomere Clusters
h) Compact Unbanded Chromosome
g) Spiralized Chromosome
f) Chromosome Bands

**Fig. IV: 5.1. Mammalian chromosome structure, from Comings DE (1977) *Chromosomes today* 6,19 Oliver and Boyd**

bound with specific proteins, the DNA-protein interactions control the access of the stain to active sites of DNA (see also Chapter 3 of Section I and Sharma AK 1975 Birbal Sahni Lect *J Ind Bot Soc* **54**:1).

## Normal karyotype

The normal human karyotype, as identified at the IV Congress of Human Genetics in 1971, consists of 23 pairs of chromosomes, arranged serially according to size, of which 22 pairs are autosomes and one pair are sex chromosomes, XX in female and XY in male. The individual autosome pairs and the XX pair are morphologically homologous while the male XY pair is morphologically nonhomologous. The autosomes can be classified into seven groups, A to G, on their general morphological characteristics, including size and position of the centromere. Each pair can be further distinguished from the other members of the same group by the different banding patterns and to some extent by the late labelling of certain regions after feeding with tritiated thymidine and autoradiography. A banding diagram has been composed for the entire karyotype, based on the patterns observed in different cells stained with either the *Q*, *G*, *R*, or *C* techniques. Each chromosome is regarded as composed of a continuous series of bands. It is divided into regions identified by specific landmarks, such as, the ends of chromosome arms, the centromeres and certain prominent bands (Fig. IV: 5.2).

## Polymorphism

A considerable amount of polymorphism is exhibited by the chromosomes even within normal individuals. Morphological variability is observed in the number and size of satellites and of secondary constrictions and the size of acrocentric chromosomes and the Y chromosome. A report suggests a relationship of extraordinary length of Y with antisocial behaviour. Satellites have been found to be present on all ten acrocentric chromosomes, but they are rarely observed on more than six in any one cell. The short arms of the acrocentric chromosomes, the satellites and their stalks exhibit considerable variation in size.

The shortening of the short arms of the chromosomes shows no apparent phenotypic effect. They are mainly heterochromatic,

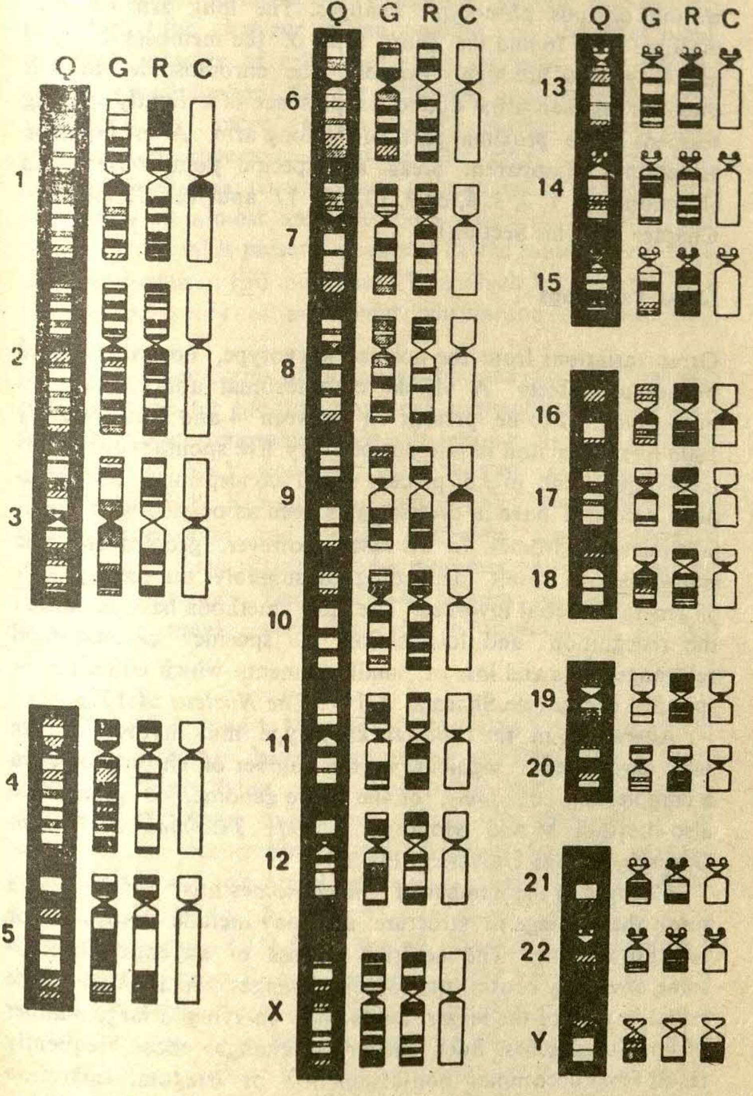

Fig. IV: 5.2. The human karyotype—diagrammatic representation of chromosomes with Q, G, R and C—banding

as indicated by the late replication of DNA in these regions, observed through autoradiographic studies. The advancements in techniques have revealed many more such variant regions without obvious phenotypic changes. The long arm of chromosome pair 16 and the short arms of the members of the D and G groups are highly variable. The chromosome No. 1 is often longer than usual due to the presence of a lightly staining segment in the proximal part of the long arm. A less common variation is an apparent break at a specific point observed in chromosomes 1, 2, 3, 8, or 9, 10, 16, 17 and the X (see also Chapter 3 of this Section).

### Gross variations

Gross variations from the normal karyotype, however, lead to phenotypic effects. A visible chromosomal abnormality has been estimated to be present in between 4 and 5 out of every 1000 live births and in one out of every five spontaneous abortions, equivalent to 3.5 percent of all conceptions. The deviations recorded have in certain cases been associated with specific clinical syndromes. In all cases, however, gross phenotypic malformations result, depending presumably on the amount of genetic material involved. The later methods have permitted the recognition and localisation of specific chromosomal rearrangements and loss of small segments which could not be specified earlier (see Sharma, A 1971 *The Nucleus* **14**:171).

Alterations in the normal karyotype may involve change in a chromosome segment, in the number of chromosomes in a complement i.e., *somy,* or the entire genome, i.e., *ploidy* (see also Levitan M and Montagu A 1971 *Textbook of Human Genetics*, Oxford University Press).

Changes in the number of chromosomes have a more severe result than change in structure and may include autosomes or sex chromosomes. The addition or loss of an entire chromosome obviously causes phenotypic changes which prove to be lethal in case of the larger autosomes carrying a large number of functional genes. Such numerical changes most frequently result from occasional non-disjunction or irregular separation of chromosomes during anaphase. If occurring during either or both of the meiotic divisions, it results in gametes with abnormal number, which may further give rise to individuals with variant complements on fertilisation. Mitotic non-disjunction, during the cleavage of an embryo, on the other hand, produces *mosaics* of cell lines with at least two different chromosome numbers. Of the different abnormalities involving one or more chromosomes, several chromosome combinations appear to be invariably lethal early in development, including: (i) nullisomy or absence of any pair; (ii) monosomy for almost any autosome, unless most of the chromosome material is present elsewhere in the same nucleus through translocation; (iii) monosomy Y in which X is absent (YO), and (iv) polysomy of autosomal chromosomes higher than trisomy.

The common forms of numerical abnormalities which survive but have a shorter life than a normal individual are: (i) monosomy for X-chromosome, XO; (ii) trisomy for sex chromosomes, XXX, XXY and XYY; (iii) trisomy for the smaller autosomes, G21, E18 and D1; (iv) double trisomy involving a sex chromosome and a small autosome; and (v) presence of two and three extra X chromosomes with or without the Y chromosomes. The effect of trisomy on development is generally more severe than that of triploidy or 45. XO monosomy.

AUTOSOMAL: Amongst the autosomes records of trisomies of larger chromosomes have been mainly obtained from aborted foetuses. Three living syndromes have been unequivocally associated with three autosomes, namely G21, E18 and D1. In abortuses the occurrence rates of chromosome types in trisomies taken as a whole are 4.7% for A, 1.3-4.5 for B, 11-16 for C, 20 for D, 30 for E, 5 for F and about 18% for G type (see De Grouchy J 1974 In: *The Cell Nucleus* **2**: 373 Academic New York; Turpin R and Lejeune J 1969 *Human afflictions and chromosomal aberrations* Pergamon, Oxford).

(i) *Trisomy G21* or Down's syndrome occurs as one in every 650 newborn. Children with this syndrome show certain characteristic features like rounded face and broad head, medial epicanthic folds, flattened bridge of nose, typically furrowed protruding tongue, together with typical mid-palmar crease, short stature and mental retardation. The first correlation of this syndrome, also known as mongoloid idiocy, with the presence of an extra chromosome, regarded as number 21 belonging to the G group, was made by Lejeune and his collaborators in 1959. The individual had a somatic set of $2n=47$ chromosomes. Later, however, about 3 percent of the patients were observed with 46 chromosomes in which a translocated chromosome with D15/G21 translocation was present. In such cases the long arm of the G21 was present three times, instead of the usual twice and therefore the extra long arm was regarded as the segment responsible for the malformations observed. Such translocations may be transmitted over several generations.

The incidence of this syndrome was observed to be related to maternal age. Mothers above 35 years of age were statistically more liable to bear mongol children. This relationship with age has been interpreted differently, the reasons given being the cumulative effects of different environmental factors including viruses on older ova or the effect of a specific gene, which results in spindle disturbance and non-disjunction.

Fluorescence studies have shown that the chromosome in G trisomy is the smaller and more brightly fluorescing one. However, since the syndrome is associated with G21, this chromosome, though shorter, retains its identity as number 21 instead of number 22.

(ii) *Trisomy E18* or Edwards' syndrome was described in 1960 by Edwards and his colleagues. The affected patients show multiple congenital malformations in almost all organs, associated with mental deficiency. The lifespan is usually very short. More females have been recorded with this syndrome than males, in about 3 : 1 ratio, possibly due to the advantage conferred in survival by the genetically more balanced XX system. Records show that there is a higher incidence of these cases with increasing age of the mother. The chromosome involved is an extra acrocentric belonging to the E group, probably a member of pair 18 as observed from morphological and autoradiographic comparisons.

(iii) *Trisomy D1* or Patau's syndrome was first associated with a chromosomal imbalance by Patau and his group in 1960. It occurs much less frequently than trisomy E 18, since the phenotypic effects are much more severe, resulting in death within a short period of birth. The syndrome is characterised by microcephaly, microphthalmos, lowset malformed ears, bilateral harelip and cleft palate; polydactyly, cardiac anomalies, accessory spleen, large gall bladder and other deformities, together with mental deficiency. It occurs in about one case out of 5000 newborns and a large proportion are females.

The trisomy, when detected morphologically, was seen to involve a member of the group D, chromosome numbers 13 to 15. It was referred to as trisomy Dl, meaning first trisomy of the D group. Autoradiographic analysis later indicated that the chromosome is D13.

(iv) *Trisomy C8* is a new syndrome identified through the use of banding patterns, characterised by mental retardation and skeletal abnormalities. Most patients show mosaicism for two cell lines, one normal and the other with trisomy C8. A record of *trisomy C9* has been described with mental retardation and abnormal phenotype.

(v) *Trisomy for partial extra chromosomes* has been identified in certain cases through the aid of two or more of the banding patterns where possibly trisomy of the full chromosome becomes lethal. Patients with an apparent extra G chromosome but *without* Down's syndrome were observed. In some such cases, autoradiography and fluorescence studies showed the extra chromosome to be either G22 or the proximal portion of D13. In another case, simultaneous use of two banding techniques showed the extra chromosome as a partial D15.

(vi) *Monosomy for chromosome G* has been recorded in about 20 cases, constituting a very heterogeneous group. In most cases they present partial monosomy resulting from mosaicism without structural changes such as 46, XX or XY/45 or XY-G, or from structural rearrangements like a ring G or deletion G.

SEX CHROMOSOMAL: Numerical aberrations involving the sex chromosomes are much more frequent in the live births. The two phenomena responsible for the survival of sex chromosomal anomalies are *dosage compensation*, through which the extra X chromosomes, above the one required for functioning, are heterochromatised or become temporarily nonfunctional and the presence of mosaics, with two or more cell lines, of which one may be normal, in a large proportion of such individuals. Sexual differentiation in man involves both genetic and post genetic sex, as given in the accompanying chart (Fig. IV: 5.3):

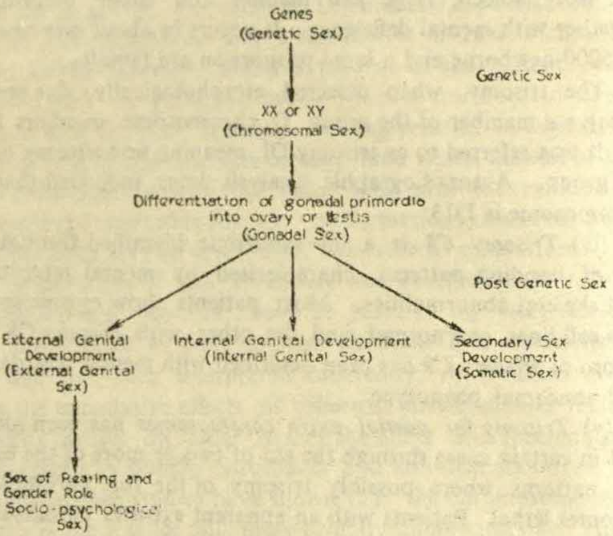

**Fig. IV : 5.3. Scheme of sexual differentiation in man (modified from Federman DD 1957 *Abnormal sexual development* Saunders, Philadelphia)**

Certain well-known syndromes have been associated with changes in the number of sex chromosomes, including: (i) monosomy for X chromosome, XO, also known as Turner's syndrome; (ii) Klinefelter's syndrome which has combinations of more than one X with at least one Y, like XXY, XXXY, XXXXY and XXYY; (iii) polysomy X, without Y; and (iv) polysomy Y, XYY, XYYY.

The nature of the syndrome can be determined by sex chromatin and Y-body analysis from the buccal mucosa and epithelial cells from hair roots. In a normal female, of the two X chromosomes, one becomes heterochromatic in the course of development and appears as a chromatin positive spot, also known as a Barr body, stainable by orcein in the interphase nucleus. In complements containing higher number of X chromosomes, the number of such chromatin positive spots increases correspondingly and gives the number of extra X chromosomes present. The Y chromosome has been shown to have a brightly fluorescent band on its long arm, appearing as a fluorescent spot in the interphase nucleus, when stained with quinacrine dyes and observed under ultraviolet light. The number of Y chromosomes and extra X chromosomes associated with different syndromes can be recognised from the number of Y spots and sex chromatin masses in a large number of the interphase nuclei (Table IV: 5.1).

**TABLE IV: 5.1**

| *No. of sex chromatin masses* | *Nature of sex chromosome constitution* | *Number of Y bodies* |
|---|---|---|
| Nil | XO | Nil |
| ,, | XY | One |
| ,, | XYY | Two |
| ,, | XYYY | Three |
| One | XX | Nil |
| ,, | XXY | One |
| ,, | XXYY | Two |
| Two | XXX | Nil |
| ,, | XXXY | One |
| ,, | XXXYY | Two |
| Three | XXXX | Nil |
| ,, | XXXXY | One |
| Four | XXXXX | Nil |

(i) *Syndrome associated with absence of X chromosome* (XO), also known as Turner's syndrome or gonadal dysgenesis, is characterised by female phenotype, short stature, sterility, retarded sexual development, webbing or looseness of skin of neck and other abnormalities. It occurs in a frequency of one in 3000 newborns. The number is much higher in abortuses. The non-disjunction resulting in the 45, XO karyotype apparently occurs after the establishment of the zygote, during mitosis resulting in cleavage. A large number of cases have been reported with mosaics, one cell line having 45, XO and the other normal, indicating that abnormal mitotic separation has been responsible for the initiation of the former. Cases have also been recorded with the symptoms of the syndrome associated with 46, XX or 46, XY karyotype. In such instances, possibly one of the X chromosomes is deficient in the former and the Y non-functional in the latter.

(ii) *Klinefelter's syndrome* occurs in one out of 500 liveborn males. It is otherwise known as testicular dysgenesis or seminiferous tubule dysgenesis. The most novel karyotype is 47, XXY but it is observed whenever more than one X occurs in the presence of a Y chromosome, including XXYY, XXXY, XXXYY and XXXXY. Mosaics of two, three, four and even six cell lines, involving these karyotypes with or without normal karyotypes, have been recorded by different workers, as also between structurally and numerically altered karyotypes. The most frequent mosaic is between XY and XXY, the XX/XXY karyotype being less common. Phenotypically, the subjects present a wide variability depending on the preponderance of normal cell lines in the mosaics, from nearly normal to severe conditions. Usually the XXY syndrome is associated with degeneration of seminiferous tubules, aspermia, feminised secondary sexual traits, long limbs, gynecomastia and sterility. Mental retardation, limited intelligence and social inadequacy are accompanying features.

The greater the number of X chromosomes present, the more severe is the mental defect. Advanced maternal age is often correlated with the incidence of this syndrome as well.

iii) *Syndromes involving X-chromosome polysomy* present variations depending on the number of extra X chromosomes. from 47, XXX, 48, XXXX to 49, XXXXX. A triple X case was first recorded in 1959 by Patricia Jacobs and her colleagues. Triple X's occur in a higher frequency than monosomy X and they exhibit minor phenotypic abnormalities. The aberrations are related to the development of the sexual traits and mental retardation and often remain unnoticed till sex chromatin or karyotype analysis is carried out. The symptoms are more severe with the increasing number of the X chromosomes. During meiosis evidently preferential segregation occurs, since none of the children, where observed, of such polysomy X mothers shows a similar polysomic karyotype or even XXY.

(iv) *Syndromes involving Y chromosome anomaly* (XYY) are rather common, the incidence being about one in 250 births. It possibly results from non-disjunction at the second meiotic division of the paternal germ cells. It can also be produced by mitotic disjunction in early zygotic development, resulting in XO/XYY or XO/XY/XYY mosaics. Conflicting data are available regarding the physical and mental makeup of patients with this anomaly. Earlier workers, including Patricia Jacobs and her co-workers in 1965, associated the occurrence of the extra Y chromosome with criminal propensities and abnormal height, based on studies on inmates of security institutions. A series of later publications on normal populations have however shown that XYY adults may be completely normal and even amongst incarcerated criminals, the crime committed is against property rather than person. A consensus of the available data indicates that major physical abnormalities do not occur regularly in XYY males, probably because the Y chromosome carries relatively little genetic material of the trait-determining type. Due to presence of the extra Y, certain developmental anomalies affecting the genitalia are present. Associated features are unusual height, probably a high gonadotrophin effect, and mental retardation with I.Q. between 80 and 95. Their offspring, where recorded, are chromosomally normal.

In addition to the well-known numerical abnormalities of sex chromosomes discussed above, other disorders of sexual development are known, which appear to have some genetic basis. In certain cases, like testicular feminisation, the karyotype appears to be a normal XY. In others, complex mosaicism in observed.

NUMERICAL POLYSOMATY AND POLYPLOIDY: Changes in the entire chromosome complement, otherwise known as polyploidy, to the levels of 3n, 4n and 5n, have been reported in aborted embryos. Triploid (3n=69) individuals, forming mosaics with the normal 2n, are occasionally able to survive till infancy. A total of 6 live-born patients with triploidy has been described. They may have resulted through digyny, dispermy or allied anomalies in fertilisation. According to a report from the WHO (1966) 17 percent of all spontaneous human abortions with abnormal karyotypes may be triploids and 7 percent are mosaics. Later reports indicate up to 60 percent presence of chromosomal imbalance in abortuses, the types of aberration varying with the time of arrest of the zygote development. The breakdown of the data on triploidy in abortuses shows proportions of XXY: XXX: XYY as 42: 25: 4, with a predominance of 69, XYY type. A common phenotypic feature of triploidy is is a characteristic swelling of the chorionic villi. referred to as hydatidiform degeneration or transitional mole. Tetraploidy is observed in about one-quarter the frequency of triploidy with both XXXX and XXYY constitutions in equal number.

STRUCTURAL: Changes in chromosome structure involve two or more breaks and rejoining with or without change in the total amount of chromatin present.

(i) *Deficiency*: The largest and most frequent deficiencies in man compatible with life are those involving the sex chromosomes. Isochromosome X has been recorded in sex chromosome anomalies. X-chromosome deficiencies are usually significant because they often give rise to *chromatin-positive* Turner's syndromes. Deletion of part of one of the short arms is associated with the full expression of the Turner's syndrome while that of one of the long arms has a less prominent expression, suggesting that the major stigmata of the syndrome are controlled by the genes on the short arm of chromosome X. Deficiencies of the long arm of the Y chromosome including the distal fluorescing segment, on the other hand, do not have any appreciable phenotypic effects, possibly because fewer genes are present on this chromosome and the male-determinant factors are located on the short arm. Certain well-known syndromes have been correlated with autosomal deficiencies. A deficiency of the short arm of chromosome B5 leads to a characteristic condition known as the Cri-du-chat or Cat-cry syndrome, first recorded by Lejeune and his group in 1963. The infant, in addition to stigmata like hypertelorism, epicanthic fold and saddle-nose, has a typical mewing cry, like that of a kitten, associated with mental and motor retardation. The condition can be inherited if the mother is a balanced carrier with a translocation between B and D chromosomes. Somewhat similar symptoms are present in infants carrying a deletion on the long arm of B5 as also on one arm of B4 chromosome, but the mewing cry is absent. Quinacrine fluorescent studies indicate the common denominator in the cat-cry syndrome to be the deletion of parts of the bright band and its adjacent distal pale band located in the middle of short arm of B5.

Partial deletion of long arm of D13 results in severe growth retardation, facial and visceral malformations and absent thumbs. Effects of deletions of D14 and 15 are less prominent.

In syndromes associated with partial deletion of the long arm of E18, the typical features are retardation in mental and motor development, microcephaly and other strikingly abnormal facial characters. Deletion of short arm of E18 also results in a typical syndrome with short stature and mental retardation.

The association of a deletion of the long arm of chromosome G21 with chronic myelogenous leukaemia (CML) was suggested by Nowell and Hungerford in 1960. The deleted chromosome was named Philadelphia (Ph$^1$). The patients are mosaics for it, since only the leucocytes carry the Ph. It is apparently acquired later in the haemopoietic tissue and is not transmitted to progeny. It is the only specific marker chromosome as yet definitely confirmed for a particular type of cancer. Quinacrine fluorescence has, however, identified the Philadelphia chromosome as G22. The chromatin material missing from its long arm has been found by banding on the long arm of a C9 chromosome.

(ii) *Ring chromosomes* are basically deficiencies and their carriers show the consequences of missing genetic material. They occur through breakage and rejoining and may involve the X or any autosome. Such chromosomes have been recorded in the acrocentric groups D, E and G but occasional rings have been found of A and C chromosomes as well. Mental retardation appears to be a common feature in all of them. Ring X chromosomes are frequently present in Turner's syndromes which are quite often chromatin-positive. A small ring of chromosome G21 has been seen to give an anti-mongoloid slant, countertype of trisomy G. Patients with ring D13 often have thumbs as opposed to polydactyly in trisomy. Rings for D14 and 15 have been also seen. Amongst the autosomes, a ring G21 can be distinguished from ring G22 with quinacrine fluorescence, both giving rise to phenotypically different conditions. Ring E18 shows a combination of the characters of the syndromes associated with deletions of long and short arms of the chromosome E18. Ring B5 exhibits characteristics similar to B5 deletion *Cri-du-chat* syndrome.

(iii) *Duplication:* Small duplications have been recorded but do not show any specific phenotypic effects. Larger duplications are more drastic. The presence of the long arm of G21 in an extra dose, when translocated on G or D chromosome, shows the same effect as trisomy G.

(iv) *Inversions* lead to rearrangements in genic material following two breaks and subsequent rejoining. Paracentric inversions, not involving the centromere, are difficult to identify since they do not result in any detectable change in chromosome morphology. A few records are available of a bridge formation during meiosis in presumed heterozygotes. Pericentric inversions have been reported in several cases, where they could be detected through appreciable change in chromosome morphology. The immediate effect appears to be reduced fertility, and a later one the birth of anomalous children, presumably because of crossing over within an inversion loop during meiosis. A pericentric inversion in chromosome C10 in a carrier female was seen to result in five births with multiple congenital abnormalities.

(v) *Translocations* involve the exchange of chromosomal segments resulting in rearrangements. The different forms of translocations have been observed in man though not related with any specific syndromes. All cases show lowered fertility. They include many possible combinations of the human chromosomes, though most frequent are interchanges between the acrocentrics or near acrocentrics. Quite common is the D/D translocation. Most significant are the cases of Down's syndrome due to translocation of nearly all of the small acrocentric G21 to an E14-15 or G21/22. Shifts and transpositions in normal individuals have been recorded by Lejeune and Berger in 1965. However aneuploid crossover products of these chromosomes result in *abnormal* progeny with similar karyotypes, a phenomenon known as "aneusomie de recombinasion".

## Comments

The causes of spontaneous chromosomal aberrations are as yet not fully clear though many chromosome breaking agents are known. They range from the X-rays and virus to a number of drugs and chemicals, most of which are of everyday use. Some of them cause breaks followed by rearrangements while others result only in breakage. Certain diseases are being identified, which apparently unrelated to chromosome structure, are associated with an increased incidence of chromosomal breakage, such as

Bloom's syndrome, characterised by dwarfism of low birth weight type and a sun-sensitive skin disorder. In addition, chromosomal breakage and leukaemia are associated in several other situations, like irradiation and benzene intoxication. A cumulative effect of exogenous factors results in the breakdown of cellular physiology, allying parental age with frequency of chromosomal changes Correlation is also observed between aging and chromosomal changes in normal individuals drawn from a random population. Increasing age in adults is associated with an increasing proportion of aneuploid cells in leucocyte cultures. Amongst women the increase becomes noticeable between 55 and 65 years of age and the chromosome most often affected is a medium sized submetacentric. In men, however, the change comes a decade later, the chromosome involved being a short acrocentric. The correspondence of these chromosomes with X and Y respectively is interesting.

Congenital defects, including chromosomal abnormalities, are mostly eliminated before birth through natural selection. They have been less affected by advances in medicine and improvements in the environment. On the other hand, innovations in medical technology have reduced early mortality, giving the surviving bearers of hereditary disorders a longer lease of life, together with a chance of propagation. Human chromosome study may be applied, with profit, in their prevention, through ascertaining those at risk, followed by treatment where possible and genetic counselling.

Disorders due to single genes and gene-environment interaction, as presented in the next chapter, are however much more numerous and the list of known conditions assigned to such causes is being continually increased (see books listed at the beginning of this chapter and Table IV: 5.2).

**TABLE IV: 5.2**

**Summary of the number of loci assigned to chromosomes at each of the four gene mapping workshops**

| | *No. of assignments recorded* | | | |
|---|---|---|---|---|
| *Conference* | *Provisional* | *Inconsistent* | *Confirmed* | *Total* |
| New Haven (1973) | 28 | 5 | 31 | 65 |
| Rotterdam (1974) | 32 | 6 | 48 | 86 |
| Baltimore (1975) | 46 | 7 | 72 | 125 |
| Winnipeg (1977) | 82 | 11 | 83 | 176 |

There are $3 \times 10^9$ nucleotide pairs in a human gamete. The figure is comparable to the population on the earth and to the duration of the human life in seconds. The range of 100,000 to 1,000,000 would seem a reasonable estimate of the number of genes. The number of metabolic pathways in which a block will lead to a serious phenotype defect, i.e., the number of recessive disorders in man, may be between 10,000 and 100,000. Most of them will be very rare or even potential. Number of recessive lethals in man has been estimated to be 0.3 to 8 per gamete and incidence of serious dominant disorders at birth 1/1000 to 10/1000.

CHAPTER IV: 6

# GENETIC DISORDERS AND GENETIC POLYMORPHISMS IN MAN

## A. Inborn errors of metabolism

At the beginning of this century, the concept of inborn errors of metabolism in man was formulated by Sir Archibald Garrod, from his classical studies on certain congenital diseases. The first one was alkaptonuria, in which the urine of the patient darkened when allowed to stand at an alkaline pH; the tissues showed grey to bluish black pigmentation and arthritis developed at a very early age. The urine, on analysis, was seen to contain large quantities of homogentisic acid, usually present in trace amounts in normal human urine. The relatives of the patient showed this disease to a certain extent. Similar inheritance of certain other diseases was also recorded, as for example: (a) albinism, in which there was a failure of pigment formation; (b) pentosuria, characterised by an increase in the pentose sugar in the urine; (c) cystinuria, with an abnormally high level of cystine in the urine. Garrod, from these studies, deduced that each one of these diseases was caused by an inherited deficiency of an enzyme required to catalyse a specific metabolic step.

Later many more diseases were added to the list, so that there are more than hundreds recorded now in which inborn errors of metabolism are involved. They range from anomalies in the amino acid metabolism, such as phenylketonuria, histidinaemia and hyperprolinaemia; to those in purine metabolism like xanthinuria and the Lesch-Nyhan syndrome; in carbohydrate metabolism like galactosaemia and glycogen storage diseases; in porphyrin metabolism; in protein synthesis like sickle cell anaemia and in lipid metabolism like Gaucher's and Tay-Sachs diseases.

Of them, two sets have been discussed in this article. Genetic disorders related to the metabolism of phenylalanine and related compounds and to lipid storage have been shown to result from improper functioning of enzyme systems involved in the different steps.

*Disorders of phenylalanine metabolism*: Phenylalanine, in man, is an essential amino acid which must be supplied in the diet. It is hydroxylated to tyrosine by an enzyme system phenylalanine hydroxylase present in the liver. Tyrosine is then converted into 3,4-dihydroxyphenylalanine (DoPA). DoPA serves as a precursor for the hormones adrenaline and non-adrenaline and the dark pigment melanin. Tyrosine is also converted into thyroxine and triiodothyronine through a series of reactions. Excess tyrosine is transformed into p-hydroxyphenylpyruvic acid through the action of tyrosine transaminase, which is further converted into homogentisic acid by an enzyme p-hydroxyphenylpyruvic acid oxidase. Homogentisic acid gives rise to maleylacetoacetic acid through the action of homogentisic acid oxidase. Maleylacetoacetic acid is successively degraded to fumarylacetic acid and then to fumaric and acetoacetic acids with the help of the corresponding enzymes. Similarly excess phenylalanine is degraded by a series of steps to compounds which include phenylpyruvic acid and phenyl lactic acids.

Five rare disorders have been associated with the lack of the corresponding enzymes in this metabolic cycle of phenylalanine and its related compounds. They are inherited as autosomal recessive traits.

(i) *Phenylketonuria* (PKU) is caused by the absence of the gene coding for the enzyme phenylalanine hydroxylase. As a result, the individuals deficient in this enzyme cannot convert into tyrosine the phenylalanine derived from the breakdown of tissue or dietary proteins. Such individuals are presumably homozygous for an inactive allele of the gene responsible for forming the phenylalanine hydroxylase system. The patients are characterised by increased concentrations of phenylalanine in the blood plasma, cerebrospinal fluid and urine. The urine also contains elevated levels of phenylpyruvic acid, phenyl lactic acid and other derivatives of phenylalanine. The clinical symptoms include nearly always mental impairment, caused by the unnatural accumulation of phenylalanine or one of its derivatives in the brain. Screening for PKU has become routine for newborns in larger hospitals since if the disease is diagnosed early and the phenylalanine in the diet severely restricted, phenylalanine accumulation can be controlled and the degree of mental impairment greatly reduced (Fig. IV: 6.1).

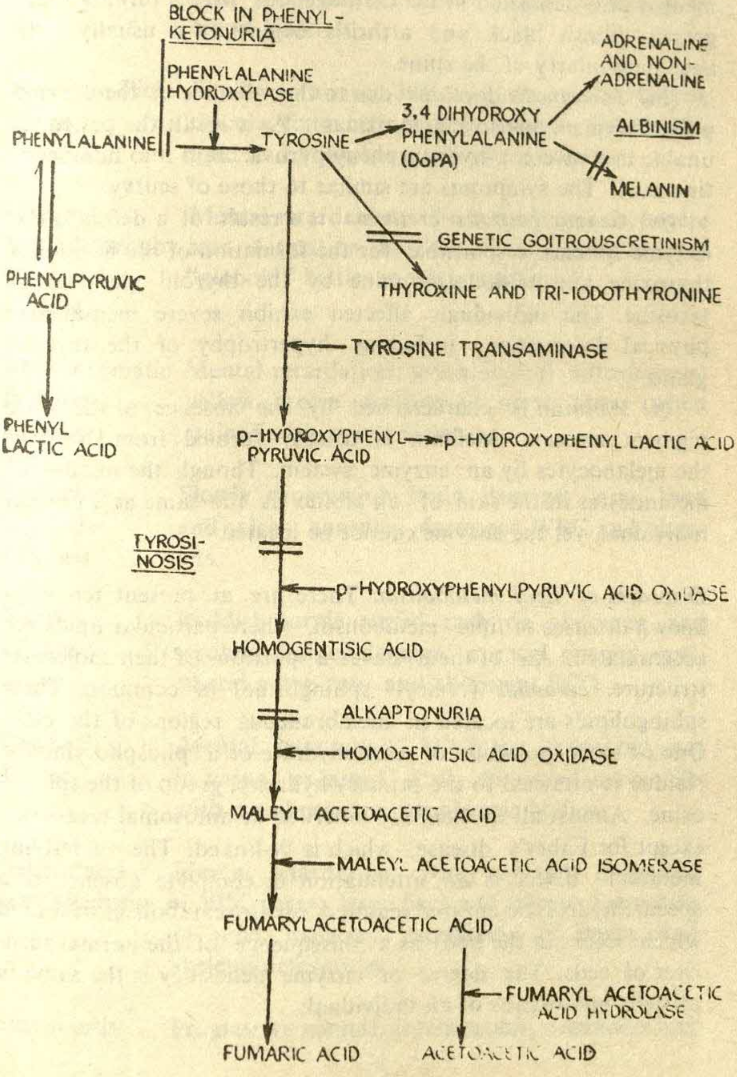
BLOCK IN PHENYLKETONURIA
PHENYLALANINE HYDROXYLASE
PHENYLALANINE
TYROSINE
3,4 DIHYDROXY PHENYLALANINE (DoPA)
ADRENALINE AND NON-ADRENALINE
ALBINISM
MELANIN
GENETIC GOITROUSCRETINISM
THYROXINE AND TRI-IODOTHYRONINE
PHENYLPYRUVIC ACID
PHENYL LACTIC ACID
TYROSINE TRANSAMINASE
p-HYDROXYPHENYL PYRUVIC ACID
p-HYDROXYPHENYL LACTIC ACID
TYROSINOSIS
p-HYDROXYPHENYLPYRUVIC ACID OXIDASE
HOMOGENTISIC ACID
ALKAPTONURIA
HOMOGENTISIC ACID OXIDASE
MALEYL ACETOACETIC ACID
MALEYL ACETOACETIC ACID ISOMERASE
FUMARYLACETOACETIC ACID
FUMARYL ACETOACETIC ACID HYDROLASE
FUMARIC ACID
ACETOACETIC ACID

Fig. IV : 6.1

(ii) *Alkaptonuria* develops in livers of individuals where homogentisic acid oxidase, which catalyses the oxidation of homogentisic acid to maleyl acetoacetic acid, is absent. The urine darkens on standing in alkaline pH due to the presence of a large quantity of homogentisic acid in it. This acid is quickly oxidised to a black pigment, alkapton. This black pigment is also deposited in the cartilagenous tissues turning them grey to bluish black and arthritis begins at a usually early age, particularly of the spine.

(iii) *Tyrosinosis* develops due to the absence of the enzyme p-hydroxyphenylpyruvic acid oxidase. As a result the patient is unable to convert p-hydroxyphenylpyruvic acid into homogentisic acid. The symptoms are similar to those of scurvy.

(iv) *Genetic goitrous cretinism* is a result of a defect in the enzyme systems responsible for the formation of the hormones thyroxine and tri-iodothyronine by the thyroid glands from tyrosine. The individuals affected exhibit severe mental and physical deformities, including hypertrophy of the thyroid gland.

(v) *Albinism* is characterised by the absence of the black pigment, melanin. Melanin is usually formed from DoPA in the melanocytes by an enzyme system. Though the number of melanocytes in the skin of an albino is the same as a normal individual, yet the enzyme cannot be located.

*Disorders of lipid metabolism*: There are at present ten well-known diseases of lipid metabolism, where particular lipids are accumulated. All of them have a portion of their molecular structure, *ceramide* (N-acyl sphingosine) in common. These sphingolipids are located in membraneous regions of the cells. One or more molecules of carbohydrate or a phosphorylamine residue is attached to the primary hydroxyl group of the sphingosine. Almost all of them are inherited as autosomal recessives, except for Fabry's disease, which is X-linked. The underlying metabolic defect is an attenuation or complete absence of a specific hydrolytic enzyme required for the catabolism of a lipid which occurs in the body as a consequence of the normal turnover of cells. The degree of enzyme deficiency is the same in all cells and organs of an individual.

| *Sphingolipidoses* | *Clinical features* |
|---|---|
| Gaucher's disease | Mental retardation (infantile); enlarged liver and spleen, hip and long bone erosion; lipid-laden cells in bone marrow; increased serum acid phosphatase; mild anaemia; decreased blood platelets. |
| Neimann-Pick disease | Similar to above; 30% with cherry red spots in macula; foam cells in bone marrow staining for lipid and phosphorus; severe emaciation. |
| Globoid leukodystrophy (Krabbe's disease) | Mental retardation; absence of myeline; severe scar formation in the brain; multinucleate "globoid" bodies in white matter. |
| Metachromatic leukodystrophy | Mental retardation; psychological disturbances; yellow brown droplets in nerve fibres when stained with cresyl violet. |
| Ceramide lactoside-lipidosis | Slowly progressing brain damage, large liver and spleen, anaemia, decreased WBC and platelets. |
| Fabry's disease | Reddish-purple raised rash on abdomen and scrotum; kidney damage; corneal opacity; peripheral nerve pain and abnormal ECG |
| Tay-Sach's disease | Mental retardation, blindness, cherry-red spot in retina, enlarged skull, distended nerve cells with membraneous cytoplasmic bodies. |
| Generalised gangliosidosis | Mental retardation; cherry-red spot in macula in 50% cases; large liver and spleen: foam cells in bone marrow; rarefaction of bones and skeletal deformities. |
| Fucosidosis | Progressive mental degeneration; weakness and |

spasticity of muscles; emaciation; thickening of skin and enlargement of the heart (Fig. IV: 6.2).

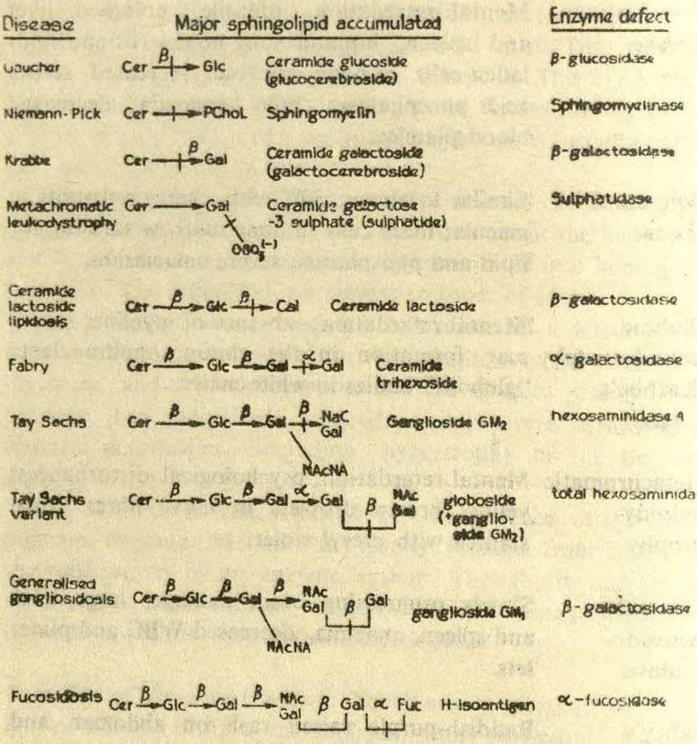

Fig. IV : 6.2 Accumulating sphingolipids and missing enzymes in sphingolipidoses (Cer = ceramide; Glc = glucose; PChol = phosphoryl choline; Gal = galactose; NAcNA = N-acetyl neuraminic acid; NAcGal = N-acetyl galactosamine; Fuc = fucose; NAcGlc = N-acetyl glucosamine). The position of normal degradation is indicated *

* Taken from Brady RO (1973) *Angew Chem Internat Edit* **12**: 1.

*Therapeutic possibilities* at present available or suggested for inborn errors of metabolism include:

(i) Enzyme replacement by organ transplantation;

(ii) Prenatal administration of purified enzymes, from human sources, human preparations or as encapsulated enzyme in biodegradable microspherules;

(iii) Percolation of blood over stably bound enzymes;
(iv) Administration of DNA through transducing viruses;
(v) Antienzyme antibodies;
(vi) Hybridization and replacement of patient's cell; or
(vii) Administration of metabolic cooperative material.

### B. Genetic control of drug effects

HISTORY: Pharmacogenetics evolved as an offshoot of human biochemical genetics in the 1950's, when attention was diverted to genetically determined enzyme variants in man which might predispose their carriers to abnormal reactions on exposure to a drug. Garrod had suggested much earlier (1909) a chemical basis for idiosyncrasies to articles of food and drug. Haldane in 1954 had predicted the application of biochemical genetics in the studies of diathesis and idiosyncrasies. In the 1950's, the basis for severe haemolysis, developed following the administration of the antimalarial drug primaquine, was discovered. This reaction was observed in about 10 percent American negroes but very few Caucasians and was also caused by other 8-aminoquinoline drugs. It was due to an erythrocytic enzyme deficiency, the G6PD, which affected the nonprotein glutathione levels of the RBC. Primaquine sensitivity was then observed to be inherited as an X-linked trait in American negroes. At about the same time, occasional patients were found to develop prolonged apnea on being given suxamethonium. These individuals and their family members showed rather low levels of serum pseudocholinesterase—an enzyme known to hydrolyse suxamethonium. Inhibition tests were then devised which demonstrated that their pseudocholinestase was qualitatively abnormal. The mode of inheritance of suxamethonium sensitivity was autosomal recessive.

In the 1960's, discontinuous distribution of isonazid blood levels was correlated with variations in acetyl transferase activity, which in turn was determined by single gene variations. These experiments, linking biochemical studies on the nature of drug reaction with genetic studies, laid the foundation of pharmacogenetics.

DEFINITION: At the present state pharmacogenetics may be defined as the field dealing with the effects of genetic variations on response to drugs (Motulsky AN 1972 in *Human Genetics* 381, Excerpta Medica). It includes, in addition, the genetic diseases which may render their carriers differentially susceptible to certain drugs as also the differential reaction of embryos to various drugs dependent upon their genotype. The formation of antibodies during drug allergies is itself under genetic control, thus coming within the scope of pharmacogenetics. The role of drugs in giving rise to point mutation or chromosomal aberrations is regarded as chemical mutagenesis but the possible genetic susceptibility to such mutagenic agents should come under pharmacogenetics. Several estimates indicate that 5 percent of all hospitalisations occur due to adverse reactions to prescribed drugs and during hospitalisation, about 15 percent patients show such reactions.

HEREDITARY CONTROL: After the initial identification of genetic variations in drug action mentioned earlier, studies showed that in many cases such reactions are not due to a single Mendelian gene. Several steps are involved in the movement of a drug through a mammalian organism, each mediated by an enzyme and therefore susceptible to genetic variation. Thus only major effects or those where a threshold mediates for all-or-no phenomena can be readily detected. Most enzyme variants are associated with only relatively small changes in the levels of activity. Twin studies have indicated that *both* heredity and environment are important in the expression of the drug effects. Polygenic inheritance has been demonstrated from family studies in many cases, including nortriptyline and phenylbutazone disposal. Occasionally, the genetic control of drug action is found to be associated with some other unrelated genetically controlled phenomenon. For example, women with blood group A are found to be three times more likely to develop thrombosis than women of other blood groups when given steroid contraceptive pills.

POLYMORPHISM: When an enzyme is tested through electrophoretic patterns, a significant proportion of individuals in a population is seen to carry a different enzyme than the standard type. This altered enzyme may be responsible for an altered level of activity so that the individual bearing it may react differently on exposure to a drug requiring the enzyme for its activity. More than 1 to 2 percent members of a population carry a gene specifying a variant enzyme (Harris H 1975 *Principles of human biochemical genetics* North Holland Amsterdam; Sharma A, Talukaer G and Mukherjee SK 1976 *Biochemical Methodology* The Nucleus, Calcutta). Allelic genes specifying gene products with gene action within a normal range are known as isoalleles. For example, in the G6PD locus, a GDA + gene product, associated with 15 percent lower enzyme activity, has been detected electrophoretically. A vast amount of work is possible on such polymorphisms. If about 15 percent of proteins are polymorphic and if the total number of genes coding for proteins in man is approximately 50,000 at least 7,500 proteins should be polymorphic.

FACTORS AFFECTING GENETIC RESPONSE: Considerable genetic heterogeneity is recorded in the loci involved in abnormalities like G6PD deficiency. The latter may be caused by many different mutations at the X-linked G6PD locus. The African type of G6PD deficiency causes milder haemolysis with drugs than the Mediterranean type. For the treatment of such genetically and biochemically heterogeneous diseases, which may otherwise be clinically homogeneous, pharmacogenetics is assuming greater importance. Racial differences in drug sensitivity have also been uncovered. In addition to the higher susceptibility of American negroes to primaquine, clioquinol or enterovioform —a common antidiarrheal agent—was found to induce subacute myelo-opticoneuropathy in a large section of Japanese populations. Since it is widely used in Europe and the USA without apparent ill effects, obviously the Japanese have a different reaction to this drug. Heterozygosity for certain inborn errors of metabolism may also lead to variant drug reaction. For example, methaemoglobinemia is a rare defect inherited as an autosomal recessive, which causes cyanosis early in life. Parents of such individuals are heterozygous for the gene and have normal methaemoglobin levels under normal conditions. When subjected to certain antimalarial drugs which induce the formation of methaemoglobin, such carriers, due to the presence of an insufficient quantity of methaemoglobin reductase, are unable to reduce the methaemoglobin and cyanosis results.

APPLICATION: Pharmacogenetics can be put to practical use in the screening of populations with a high frequency of traits predisposing to untoward drug reactions. Racial distribution of such traits should be considered prior to preparing suitable screening programmes. G6PD deficiency is very rare amongst northern populations, quite common amongst negroes and not infrequent in Mediterranean populations. Similarly pseudocholinesterase abnormality is absent in negroes and very rare in the Japanese.

It has a further role in ecogenetics or the study of genetic aspects of ecology. Toxic agents present in the environment may damage certain genetic constitutions preferentially or alternatively render them susceptible to damage. In testing the effects of different drugs to obtain an indication of the genetic susceptibility to them, different test systems, as described in Chapter IV:9 may be used.

### C. Effects of antibiotics

Antibiotics are frequently used to counteract bacterial infections in the human systems. Some of them have been analysed thoroughly and they are observed to affect the synthesis of proteins or nucleic acids in the bacteria.

Antibiotics like mitomycin C and actinomycin D interact with the nucleic acids, showing very specific activity. Mitomycin C, which has both antitumour and antibacterial properties, affects the synthesis of DNA but RNA and protein synthesis continues unabated till the reserves are exhausted. DNA is apparently damaged through crosslinking and replication, depending on DNA-dependent-DNA-polymerase, is hampered. Transcription is not affected.

Actinomycin D, on the other hand, terminates RNA synthesis, though replication and protein synthesis are initially unaffected. The chemical forms a reversible complex with DNA, preventing transcription and blocks it as a primer for DNA-dependent-RNA-polymerase (DDRP). Its action, both as inhibitor and antibiotic, can be reversed by adding DNA. It complexes best with double-stranded DNA containing guanine and is insensitive to artificial polynucleotides without guanine. Therefore actinomycin D is suggested to bind to DNA in the narrow groove of the double helix. It affects DDRP at only much higher concentrations, since DNA is mostly unwound before replication and actinomycin D binds poorly with single-stranded DNA.

DNA is the primer for both DNA and RNA polymerases. For production of DNA polymerase, the two strands of the double helix separate to replicate, while for the production of RNA polymerase, they may remain as a duplex, explaining the action of mitomycin and actinomycin respectively. A third type of binding with other compounds is seen to impair nucleic acid metabolism, that is, with compounds like acridine, proflavine and ethidium. In such bindings, flat cubes of the compound are inserted within the stacked base pairs, which interfere with the helical structure, extending it and interfering with the primary functions.

Other antibiotics interfere with protein synthesis, acting at the level of translation. They are more specifically active against the bacterial system and less harmful to higher organisms due to the smaller size of the bacterial ribosomes. Of them, streptomycin blocks the transfer of aminoacids from *t*-RNA molecules into growing polypeptides in the sensitive ribosomes, since the antibiotic binds at the same site at which *m*-RNA is bound. Puromycin acts both *in vivo* and *in vitro*. It resembles aminoacyl-t-RNA in structure and inhibits the transfer of aminoacids from the *t*-RNA to the growing polypeptides on ribosomes. Thus incomplete polypeptide chains are released.

Chloramphenicol, on the other hand, interferes with protein synthesis at the ribosomal level and appears to freeze polypeptide synthesis, preventing the formation of new peptide bounds.

Thus mitomycin C and actinomycin D act as complements to the natural structure of DNA, rendering the latter vulnerable. The other three compounds inhibit protein synthesis. The ultimate result is the inhibition of cell division, mitomycin C having a more immediate effect than actinomycin D. The action of such antibiotics is however directed against the bacterial system while the higher organisms are unaffected at controlled doses.

### D. Genetical polymorphisms

As mentioned earlier, in the human beings about 15 per cent of the genes coding for proteins are polymorphic. Under optimal environmental conditions, these polymorphic genes impart genetic variability and account for the widely divergent phenotypes observed. The genetic variability between sibs is 86% while that between populations of the same race 8% and between different races 6% only. Thus *Homo sapiens* is genetically a single species and the interracial differences are very minor as compared to interindividual differences.

A major factor in the manifestation of the phenotypic variability is the gene-environment interaction. Studies from lower organisms have indicated that greater polymorphism in the genetic complement is directly proportional to greater variability of the environment. For example, electrophoretic analysis of the products of about 30 polymorphic genes in Anurans shows a direct correlation between genetic and environmental variability described earlier.

These experiments, though limited, indicate the close relationship between genetic variability and adaptation to environmental diversity.

CHAPTER IV: 7

# GENETICAL BASIS OF MALIGNANCY

During the onset of many types of cancer, a single cell may undergo one or more permanent hereditary changes. The uncontrolled, rapid proliferation of this cell, resulting in millions of such altered cells leads to cancer. Boveri, more than 50 years ago, first suggested chromosomal changes as a cause of malignant neoplasms. It remains a fact that in many cancers, chromosomal alterations are a ubiquitous feature.

A malignant cell shows uncontrolled cell proliferation without any differentiation. Various theories have been put forward to explain the phenomenon. The cell develops also the property of invasiveness so that a cancerous tissue invades other tissues through a loss of contact inhibition. Such cells in culture do not form a plate-like growth as normal tissues.

This transformation of an initially normal cell into a potentially cancerous one involves presumably a change in its hereditary DNA thread, whether through change in the thread itself, or through chromosomal proteins or through irregularities in chromosome movement.

## *I. CHROMOSOMAL CHANGES*

In cancer cells, the chromosomes can undergo structural changes, both *qualitative*, like rearrangements of chromosome segments, or *quantitative*, like loss or addition of chromosome material. Changes in number may involve one or more chromosomes as well as as entire complements. Electron microscopical studies have permitted the analysis of the ultrastructure of chromosomes. In human cancer cell lines, alterations have been seen both in the ultrastructure and in the DNA packing or arrangement within chromosomes in cancer cells.

The immense amount of data collected on cancer-associated chromosomal aberrations has shown a number of correlations between chromosome change and neoplastic disease. A recent catalogue (Mitelman F 1984 Catalogue of chromosome aberrations in cancer *Cytogenet Cell Genet* **36**: 1 S Karger Basel) has shown that out of 3,844 cases of neoplastic disorders, the maximum has shown an association with chromosome number 8 (1,083) and the minimum with X chromosome (186).

### Alterations of karyotype

HUMAN CONGENITAL DISEASES AND CANCER: A very common human chromosomal anomaly is Down's syndrome, in which one extra chromosome No. 21 or its long arm is present in addition to the normal 46. Leukaemia is also often found in such individuals 3 to 20 times more frequently than expected. Usually in such individuals, only one extra No. 21 chromosome is present, but occasionally additional chromosome anomalies are found. In three more syndromes, Bloom's syndrome, Fanconi's anaemia and ataxia telangiectasia, there is an unusual fragility of the chromosome arms. As a result, chromosome breaks occur with a high incidence of chromatid gaps, isochromatid breaks, abnormal chromosomes, fragments and quadriradial figures.

There is an overall prevalence of malignancy in individuals possessing cells with increased chromosome fragility, particularly if accompanied by immunological incompetence.

ANEUPLOIDY IN HUMAN CANCER CELL POPULATIONS: The parent cell of any cancer has 46 chromosomes. Later by a series of abnormal divisions, the cancer cells contain series of chromosome numbers and karyotypes. Earlier pathocytologists, like Makino, had suggested that in different cancer cell populations there are chromosomal "stem" lines, involving a particular spectrum of chromosome structure and number and that they arise by systemic evolution of the karyotype. An established cancer cell population will have a modal number in most of the cells over quite long periods and it is relatively stable. Generally speaking, no two karyotypes are identical in cancer or leukaemia and no typical chromosome group has been found to be involved.

In solid tumours, the modes are usually between 62 and 85 chromosomes, i.e., the 3x range. They can be transplanted to other hosts and give rise to a variety of other numbers. Aneuploidy, with many supernumerary chromosomes that lack any definite pattern, characterises solid human tumours. In acute leukaemia, it occurs in only 50% of the cases. The occurrence of any aneuploid cells in normal bone marrow foreshadows leukaemic attack. After chemotherapy, these cells decrease sharply. Their increase predicts a relapse.

### Structural alterations of chromosomes

MARKER CHROMOSOMES: Marker chromosomes of a structure different from the normal ones are found frequently in human cancer cells. Their number in each cell is usually 2 or 3. If such chromosomes are found in all cells of a particular tumour, it indicates its origin from a single initial cell.

*Philadelphia (Ph′) chromosomes*: Chronic myelocytic leukaemia is characterised by increase in WBC (over 50,000 per mm$^3$) and proves fatal within a short time. The chromosome specific for this disease is a G type one with a portion (about 40 per cent) broken off from the long arm. It is referred to as Philadelphia or Ph′ chromosome. Banding pattern analysis has shown this chromosome to be often formed by a reciprocal translocation of segments between chromosomes 22 and 9. Ph′ occurs in all blood cell lines but not in skin fibroblasts. In CML, it is present alone in the earlier phases but is associated with other unspecific abnormalities in the acute terminal phases. Often a clonal evolution can be observed in these cell populations, starting from Ph′ and progressing to successive acquisition or loss of supernumerary chromosomes, specially in groups 21-22, 17-18 and 6-X-12. Different chromosomal translocations have been described in other tumours as well, depending on the type of tumour. For any one tumour, however, the chromosomes and even the exact breakpoints are almost always the same.

*Large acrocentric chromosomes in solid tumours*: In such solid carcinomas, usually large acrocentrics of the same size as A, B and C types are observed. Occasionally rings or dicentrics may also be present. In most cases of Burkitt's lymphoma, a large acrocentric marker has been observed. However they are not as consistent as CML. Apparently in solid tumours of very different origin, the same markers can be found. The reason for the origin of acrocentric markers is possibly their greater survivability in the rapidly dividing cancer cell. In the formation of such markers in Burkitt's lymphoma, usually chromosomes 1, 2 and 3 are involved. The marker chromosomes are possibly formed as a secondary event following the still unknown alterations changing a normal cell into a cancerous one. They point to a single cell origin for most human tumours.

*Other structural changes*: The chromosomes of cancer cells *in vivo*, when compared with the normal ones, exhibit general structural disturbances. Even from the same individual, the chromosomes of leukaemic cells are fuzzy, ill-defined, irregular, with poor separation of chromatids. These general disturbances can barely however be recognised under light microscopy.

ALTERATIONS IN CHROMOSOME ULTRASTRUCTURE: Whole mount electron microscopy shows certain features in Burkitt's lymphoma. The large marker chromosome has an attachment with a pair of acrocentric D chromosomes by a few mechanical fibres. It however does not have any gross difference in its fibrillar composition from a normal chromosome. The absolute dry mass of malignant and non-malignant chromosomes, when measured from different preparations, did not show any significant difference. The main structural element of a chromosome is a long irregularly folded fibre. It is not straight with a regular diameter but bumpy like a twisted spaghetti. Dry mass determinations show that the DNA is very tightly arranged within the chromosomal fibre. The high packing ratio of DNA in chromosome fibres is only possible by supercoiling. The ratio varies in individual chromosomes, depending upon the degree of contraction. However, the ratios are usually greater than 100 : 1 in fibres from normal chromosomes and not so high in the marker chromosome in Burkitt's lymphoma. It is possible that chromosome fibres from malignant cells are altered and that the changed relationship of DNA to protein disturbs their elastic behaviour, leading to fibre breakage or altered folding patterns.

## *II. INDUCTION OF CANCER*

Chromosome changes can be induced *in vitro* and *in vivo* by ionising radiations, chemicals and viruses. Each of these factors can also transform normal cells into malignant ones.

### A. VIRAL CARCINOGENESIS

The inducing factor in *viral carcinogenesis* is the viral DNA, which persists in the transformed cells and is integrated in the host chromosome of the cancer cell. The retention of this part of the viral genome in the cancer cell causes its persistent chromosomal instability. Herpes-type viruses have been detected in Burkitt's lymphoma and acute anaemia. They are able to transform normal human leucocytes *in vitro* and also to produce chromosomal changes ranging from breaks, secondary constrictions, polyploidy to final pulverisation.

The frequently accepted idea about viral carcinogenesis is that the viruses are responsible for the ultimate development of cancer, the physical and chemical agents acting as co-carcinogens. It postulates the presence of *virus suppressor loci* on chromosomes which may, on being subjected to external agents, mutate permitting the virus to act in inducing malignancy. For example, immune suppressor agents are known to alter the responses of immune-responsive cells.

Several *oncogenic* (cancer-producing) viruses have been associated with specific cancer as for example, (i) *RNA viruses:* mammary cancer, sarcoma and leukaemia viruses in mouse and avian leukosis viruses, and (ii) *DNA viruses*: Simian virus 40 (SV 40); polyoma, papilloma and herpes viruses, all acting on different mammals; and adenoviruses.

The DNA virus Epstein Barr (E-B) is claimed to be the causal organism for Burkitt's lymphoma amongst East African children and also for naso-pharyngeal carcinoma restricted to South-East Asia. In certain cases, geographical factors may act as a triggering mechanism for the activation of such viruses.

Of these, the herpes simplex viruses have about 200 genes and adenovirus, responsible for eye cancer, enough DNA for 55 genes. Nucleic acid hybridisation studies have shown the integration of parts of DNA viruses into eukaryotic chromosomes. For example, human lymphoblastoid cell lines are known to contain DNA homologous to the E-B virus. Radiolabelling has confirmed the integration of DNA of adenovirus AV 12 into the DNA of Chinese Hamster cells. Many, if not all, cancer viruses can exist as DNA particles, later being integrated into the host genome for subsequent transmission to daughter cells.

On the other hand, RNA tumour viruses or *retroviruses* are animal viruses consisting of a single stranded RNA molecule. The first one, Rous sarcoma virus, was isolated from a chicken tumour—and was capable of inducing tumours in other chicken. Retroviruses have also been isolated from other chicken tumours and from mammalian tumours, including human. These may be classified into two groups: (i) The first causes tumours with high efficiency after short incubation periods. These are *able to transform cells in culture* but are *unable to replicate, with the single exception of Rous virus.* (ii) The second group causes tumours with low efficiency and only after long incubation periods. It is able to infect cells in culture but *not* transform them. It can replicate leading to infected cells to shed infectious virus particles. The basis for this difference in behaviour is genetical. When a cell is infected by an oncogenic virus, a viral enzyme, *reverse transcriptase* acts as a catalyst in the transcription of a DNA copy of the viral RNA genome. This DNA is inserted randomly into a host cell chromosome. RNA molecules are transcribed from the chromosomal DNA and act both as messengers coding for proteins and as genomes to be packaged into new viral proteins and released from the infected cell.

In Rous virus, the tumour-causing property is due to a single viral gene, *src*. Viruses carrying the mutants of *src* gene infect chicken cells and replicate normally but do not produce tumours, due to the mutations. However, mutants of any of the other three viral genes isolated (*gag*, *pol*, *env*) cannot replicate but can transform infected cells. The transforming *oncogene* codes for a protein approximately 60,000 daltons in mass. This protein can act both *in vitro* and *in vivo* as a protein kinase, adding phosphate groups specifically to tyrosine residues. The other viruses of the same group, however, cannot replicate since they lack parts of the *gag*, *pol*, and *env* genes. But all of these carry an oncogene capable of transformation. The proteins encoded by these genes differ from each other and from the Rous protein.

Following *in situ* hybridisation experiments and genetic analysis of somatic cell hybrids containing chromosome fragments, a number of oncogenes have been identified and assigned to the respective chromosomes in man and mouse (see Table IV: 7.1). These two mammalian systems have been subjected to intensive genetic analysis, the first because of the anthropocentric nature of cancer research and the second due to the ease in handling. The two systems are remarkably similar in certain respects, like immune function and tumour biology.

It has been suggested that the oncogenes, in the course of evolution, first arose in cells and were then transmitted *outside the cells* by the retroviruses. Using recombinant DNA molecucules corresponding to viral oncogenes as probes, it was found that in all cases, a normal host cell DNA sequence homologous to the oncogene can be identified. The two sequences—obtained from the uninfected host cell and the viral oncogene—are similar but not identical since the latter may lack certain protein encoding segments of the former. The cellular oncogenes are apparently ancient in the evolutionary scale and unconserved since sequences homologous to mouse oncogenes have been identified in man and homologues of *src* and *abl* oncogenes located in *Drosophila*. Many of these are expressed in the normal host cells in particular cell types and at particular stages of development. In general, oncogenes, even those possibly derived from a single ancestor, are widely dispersed on different chromosomes, unlike the gene families like alpha-globins or major histocompatibility complex, where many genes are clustered at single chromosomal loci. It is possible that the overexpression of a cellular oncogene, induced by the accidental integration of a retrovirus next to it, may lead to malignant transformation of the host cells.

The retroviruses of the second group however do not depend on the presence of an oncogene for their activity. They replicate normally and are transferred to other cells by infection.

TABLE IV : 7.1

**Cellular oncogenes in humans and mice (after D'Eustachio P 1984 *Am Scientist* 72 : 327)**

| | *Human* | | *Mouse* | |
|---|---|---|---|---|
| *Gene* | *Chromosome* | *Associated tumour*$^{a}$ | *Chromosome* | *Associated tumour*$^{a,b}$ |
| *ras*$^{N}$ | 1p31.1→p22.1 | various myeloid tumours (?) | — | — |
| *skl* | 1q12→qter | — | — | — |
| *fos* | 2 | Burkitt lymphoma (?) | 12 | — |
| *raf-1* | 3 | — | — | — |
| *raf-2* | 4 | — | — | — |
| *fms* | 5q34 | — | — | — |
| *myb* | 6q22→24 | acute lymphocytic leukaemia, etc. | — | — |
| *ras*$^{K}$*-1* | 6p32→q12 | acute lymphocytic leukaemia, etc. (?) | 6 | — |
| *erbB* | 7pter→q22 | — | — | — |
| *mos* | 8q22 | acute myeloblastic leukaemia | 4 | — |
| *myc* | 8q24 | Burkitt lymphoma | 15 | plasmacytoma |

| | | | | |
|---|---|---|---|---|
| *abl* | 9q34 | chronic myelogenous leukaemia | 2 | — |
| $ras^{H}$-1 | 11p13 | Wilm's tumour | — | — |
| *rasK*-2 | 12p12→pter | — | — | — |
| *fes* | 15q22→q25 | acute promyelocytic leukaemia | — | — |
| *erbAI* | 17p11→q21 | — | — | — |
| *src* | 20 | — | — | — |
| *sis* | 22q11→qter | Burkitt lymphoma, chronic myelogenous leukaemia | — | — |
| $ras^{H}$-2 | X | — | — | — |

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[a] All associations, except that of the *myc* gene, are based on the correlation between the map position of a gene and the existence of a cytogenetically defined chromosomal rearrangement.

[b] Oncogenes homologous to *fos*, *mos*, *abl*, $ras^{H}$ and $ras^{K}$ are all found in retroviruses capable of causing tumours in mice; however only *myc* is associated with tumour formation in the absence of viral infection.

When integrated into a host cell genome, they are transmitted to successive cell generations as harmless gene loci. These are rendered harmful when a vital host gene is disrupted by the integration or through events like recombination or faulty transcription, by which a recombinant viral genome may incorporate a cellular oncogene.

*Oncogenes* are thus now known to be *eukaryotic* genes, widely dispersed in the genome and well conserved. They can be transferred into the genome of a retrovirus or activated *in situ* by the adjacent integration of a retrovirus. Recent evidences from somatic cell genetics show that these cellular oncogenes can play a part in the occurrence of *spontaneous* human tumours in the *absence* of detectable exogenous viruses. An example is the study of mouse plasmacytomas or tumours of the plasma cells, the differentiated cells responsible for immunoglobin secretion and normally incapable of division. When transformed, these cells continue to produce immunoglobulin but divide uncontrollably. The mouse plasmacytomas contain a translocation between chromosome 15 and either chromosome 12 or chromosome 6. Banding pattern analysis shows that the breakpoints on chromosomes 12 and 6 are on those particular chromosomal bands which contain the genes for the heavy and light chains of immunoglobulins, respectively. In chromosome 15, the breakpoint has been located at the site of the *myc oncogene*, sometimes even *within* it. The *m*-RNA and its corresponding polypeptide produced from the *myc* oncogene appear to be very similar to that found in the original *myc* retrovirus.

In Burkitt lymphoma, a human tumour of B lymphocytes, translocations between chromosomes 8 and 2, 14 or 22 have always been identified. Banding pattern analysis shows the breakpoints to be constantly located at the sites of immunoglobin gene clusters encoding *K* light (chromosome 2), heavy (chromosome 14) or λ light chains (chromosome 22). On chromosome 8 side, the break is at or near the human *myc* gene. The *myc* gene is transcribed into RNA at elevated levels.

Therefore, for transformation, a chromosomal translocation must not only alter an endogenous oncogene to a malignant form but also activate it so that it is expressed at a high level. This conclusion is specially important in view of the fact that specific translocations affecting chromosomes that carry oncogenes have been related to specific tumours (Table IV: 7.1)

## B. CHEMICAL CARCINOGENESIS

An interesting speculation about *chemical carcinogenesis* is that a carcinogen interacts with cellular constituents like protein RNA and DNA. These interactions lead to the critical event of initiation of a somatic mutation that confers a slight growth advantage on a target cell. This event escapes repair and is transmitted to daughter cells. Cell division may stop or continue, depending upon the renewal rate of the tissue and impinging growth stimuli (cocarcinogens, hormones or wound repair). If the stimuli are withdrawn, carcinogenesis may be interrupted. If cells continue to divide, the initial growth advantage is expressed, fixed and amplified. Clonal growth of initiated cells reaches histologic proportions and becomes visible. The cell surface undergoes modifications that can be related to the new pattern and potential of growth; the cells exhibit a spectrum of new morphological and biochemical characteristics. Promoters and other environmental factors, acting upon cells with an altered genome, cause further mutations leading to dysregulations in other genes. Successive selection of new phenotypes leads to progressive emancipation from growth control and from immune defence. The successful competitors become capable of autonomous and invasive growth.

A number of chemicals has been shown to significantly increase the incidence of malignant neoplasms. These have been termed collectively as carcinogens, though their modes of action may be different (Table IV:7.2). The development of cancer is known to be a multicausal, multistage process, involving interactions between inherited and noninherited risk factors. The first stage, that of *initiation*, may involve several stages—(a) metabolic conversion of a procarcinogen into an electrophilic or reactive form; (b) reaction of the reactive form with a critical cellular target, usually DNA; (c) subsequent action by DNA repair or replication processes by which the fate of the chemical lesion is determined and (d) conversion of the chemical lesion into a persistent biological change of the cell (see also chapter on molecular basis of mutation).

**TABLE IV : 7.2**

**Hypothetical classes of carcinogenic chemicals (ICPEMC Publ no 9, *Mutat Res* 133 : 1, 1984)**

| | *Type* | *Examples* | *Properties, possible action* | |
|---|---|---|---|---|
| 1. | Direct acting | Ethylene imine, bis (chloromethyl) ether | Electrophile, organic compound, covalent binding to DNA | Genotoxic |
| 2. | Procarcinogen | Vinyl chloride, benzo (a) pyrene, 2-naphthylamine, di-methylnitrosamine | Requires conversion through metabolic activation by host or *in vitro* to type 1 | |
| 3. | Inorganic carcinogen | Chromium | Leads to change in DNA by selective alteration in fidelity of DNA replication | |
| 4. | Solid-state carcinogen | Asbestos, plastic film, fibres | Exact mechanism unknown; usually affects only mesenchymal cells and tissues; physical form vital | Usually non-genotoxic but may also be indirectly genotoxic |
| 5. | Promoter | Phorbol esters, phenols, bile acids, anthralin, saccharin | Not carcinogenic by itself, but enhances effect of type 1 or 2 agent when given subsequently | |
| 6. | Hormonally active substances | Estradiol, prolactin, amitrole | Alters endocrine system balances and differentiation; often acts as promoter | Non-genotoxic |
| 7. | Co-carcinogen | Pryene, catechol, ethanol, n-dodecane, $SO_2$ | Not carcinogenic by itself, but enhances effects of type 1 or 2 agent when given at the same time. May modify conversion of type 2 or type 1 | |
| 8. | Immuno-suppressor | Anti-lymphocytic serum | Enhances development mainly of 'virally induced'; transplanted,or metastatic neoplasms | |

Modified from Weisburger TH and Williams GM (1981) *Science* **214**, 401.

DNA alteration is usually presumed to be a critical event in the initiation of cancer due to the observations that (a) many agents which induce cancer also induce mutations; (b) deficiencies in DNA repair predispose cells to neoplastic conversion and (c) changes in cells leading to cancer are usually irreversible and permanent; indicating that these are of a heritable nature.

In the next step of carcinogenesis, *promotion*, there are permanent changes in the cellular behaviour, so that tumours are formed showing the properties of uncontrolled growth and loss of contact inhibition. This stage is less understood than the initiation aspect. Each of these stages of carcinogenesis may be modified by a variety of agents (see also Fox H and Brennard J 1980 *Carcinogenesis* **1**:745 and Clayson DB 1980 *Mutat Res* **75**:205).

A thorough knowledge of the molecular events controlling cell differentiation and gene regulation is however required to understand the initiation of cancer.

## C. FUTURE PROSPECTS

The involvement of chromosomes in carcinogenesis still poses certain problems regarding the mode of action. The most obvious ones are the roles of oncogenes in the normal cells and the mechanisms by which oncogene mutation or overexpression can lead to malignant transformation of a cell. The exact number of oncogenes is not also known though classical cytogenetics, coupled with somatic genetic analysis and recombinant DNA technology, are yielding new information. Many different tumour-specific chromosomal translocations affect the same few oncogenes. On the other hand, certain tumours have not been associated with chromosomal translocations or mutated oncogenes or ability to transform mouse 3T3 cells in culture.

In general, the progression from a normal cell to a malignant tumour is a long process in human beings, often involving many steps. The mutation or the chromosome rearrangement that activates an oncogene or alters its product is obviously a crucial step in some forms of tumour, which is irreversible. However, the necessity of oncogene activation or its relation with the other steps is not yet known for all forms of tumour.

CHAPTER IV: 8

# GENETICS AND SENESCENCE IN MAN

Ageing or senescence is regarded to occur from fertilization onwards. Initial peaks associated with gaining of maturity and biological fitness in the sense of reproductive power and resistance to disease, accidents and predation have been visualised by some workers, followed by deterioration throughout the life span. This process is characterised by its occurrence in all members of a population. It is progressive and eventually harmful; irreversible under usual conditions, and accompanied by alterations in functional capacity which result in the loss of the organism's ability to respond to environmental changes.

Definitions of ageing have been numerous and varied and often stress different aspects of the process. Biological ageing has been regarded as the gradual decrease in the adaptation of an organism to its normal environment, following the onset of reproductive ability. There is a corresponding decrease in the ability of the organism to carry out various specialised functions, resulting in an increased probability of death with time. Medawar PB (1952 *An unsolved problem of biology* Lewis London) emphasised the physical changes associated with ageing, which render the individual progressively more likely to die from accidental causes of random incidence. Comfort (A 1968 *Nature* **217**: 320) regarded it as the total effect of all changes which occur in a living organism with increasing chronological age and which lead to greater vulnerability or lower viability.

## Genetical theories of ageing

Many hypotheses have been advanced to account for the cause and mechanism of senescence. Essentially genetical theories of ageing have been put forward from time to time, although ultimately all comparatively recent ideas in senescence imply directly, or indirectly an innate or built-in control (see Barrows CH and Kikkonen GC 1977 In: *Adv in Nutr Res* ed Draper H, 4 Plenum, New York; Cutter RH 1977 In: *A new look at biological ageing* ed Behnke *et al.* Amer Inst of Biol Sci Publ; Sharma A 1971 *Sci Cult* 37: 274 for review). There is probably no single cause of ageing. A phenomenon that may come closer to a unifying theory consists of concepts based on progressive genetic instability as a factor contributing to senescence (Hayflick 1976 *New Engl J Med* **295**: 1302).

The principal controversy involves two aspects of the process: (i) whether the changes in the genetic material are induced or programmed and (ii) whether the control is nuclear or cytoplasmic.

Most gerontologists agree on a general hypothesis that ageing is a cellular information loss, probably in fixed cells and originally at the molecular level, other effects—arising through loss of neuronal, endocrine and immunological information—being secondary. The loss of molecular information may be caused in two ways, which are not mutually exclusive: (a) it may arise as a result of the random accumulation of chemical noise in the cellular information system or, (b) it may be an inherent byproduct of differentiation, brought about by the stoppage of synthetic processes, which cannot be started again without the accumulation of late-acting harmful genes during evolution. To the former series belong the theories implying somatic mutations, at nuclear or cytoplasmic levels, while those stressing genetic regulation include the concepts of programmed senescence. The devices used by organisms for protection against chemical noise, postulated in the first series to be repair enzymes, are, however, also genetically determined, perhaps as a part of a programme to prevent premature ageing.

## Theories involving accumulation of induced damage

The *somatic mutation* set of theories postulates that ageing is due to a steady accumulation of random and permanent forms of cell damage starting at a molecular level. A modification by Strehler (SZ 1978 *Time, Cells and Ageing* Academic Press New

York), assumes both stochastic and genetic causes of ageing and regards *inborn* errors in the genotype as well as somatic mutations to contribute to the latter. These theories received an initial support from the resemblance of radiation-induced changes in mice with age-induced ones, but were challenged when certain well-known mutagens were found to be unable to produce radiation-like life-shortening even with drastic doses, unless they were also capable of causing more extensive damage than point mutation, particularly cross-linking between two strands of DNA. Another limitation has been its failure to explain the specificity of the life span of a particular species, which must be determined by some genetic factor and is an evolutionary product of natural selection (Dobzhansky Th 1958 *Ann NY Acad Sci* **71**: 1234). In refuting this argument, however, it may be suggested that since mutation rates are genetically determined, that for a particular species may be regarded as genetically controlled as well.

In a later, *error catastrophe* model, ageing is regarded as the result of random collection of errors during protein synthesis (Orgel LE 1973 *Nature* **243**: 44). It visualises that most abnormal proteins, arising through errors in the DNA skeleton or through mistranscription or mistranslation, would be diluted out by the continual turnover and replacement by newly synthesised error-free molecules. However, an error in the protein-synthesising system in the cytoplasm could be self-perpetuating and produce new errors continually. An accumulation of such errors would lead to the irrevocable breakdown of the system and corresponding loss of functioning and death of the cell. This concept received considerable support from several experiments. The longevity of *Drosophila* adults was found to be reduced following nurturing the larvae on non-toxic concentrations of amino-acid analogues. In *Podospora anserina*, feeding with such analogues led to shortening of the life span (Holliday R 1969 *Nature* **221**: 1224), presumably through incorporation into the proteins.

In addition to mice, damage to DNA by irradiation was found to be associated with decreased life span in man, insects, *Paramoecium* and cultured fibroblasts. In a number of mammals, the life span may depend on the amount of DNA repair after UV-irradiation (Hart RW and Setlow RB 1974 *PNAS USA* **71**:

2169), Diverse agents were postulated as intermediaries in the accumulation of such errors and their role in initiating age changes.

According to another modification, the *free radical theory* of ageing, normal cellular function is ultimately limited by peroxidative damage which decreases the organism's ability to respond adaptively to environmental change. Such degradative changes are caused by free radical reactions that are universal in living organisms (see Tappel AL 1973 *Fed Proc* **32**: 1870).

Burnet (FM 1974 *Intrinsic mutagenesis—a genetic approach to ageing* Wiley New York) suggested that since senescence is largely the result of somatic mutations mediated by autoimmune processes and influenced by the progressive weakening of immunologic surveillance, thymus may have a strategic rôle in controlling senescence. Later, he suggested that the frequency of somatic mutations is determined by *intrinsic factors*, like precision of key enzyme systems.

The *chalone* theory (Bullough WS 1971 *Nature* **229**: 608; Maugh TH 1972 *Science* **176**: 1407) postulates that cell proliferation is controlled by endogenous mitotic inhibitors known as chalones through negative feedback inhibition. The action is reversible and depends on the state of cellular membranes. They are tissue-specific but not species specific and are synthesised by the mature cells of the tissue on which they act.

Other indirect evidences for possible role of errors, cytoplasmic or otherwise, in ageing, are provided by the transmission of the determinant for type B *life spanning* in *Amoeba proteus*, which causes eventual death of a whole clone, to a normal clone from *spanned* amoebae by injecting the cytoplasm. In two fungi a correlation was observed between accelerated senescence and increased errors in protein synthesis. In older nematodes, both active and totally inactive enzyme molecules have been recorded by immunochemical techniques, indicating the possible role of the synthesis and collection of non-functional protein molecules during ageing (Gershon H and Gershon D 1973 *PNAS USA* **20**: 909). A deficiency of vitamin E in the diet of albino rat was seen to result in the deposition in the adrenal glands of a fluorescent pigment, apparently identical with lipofuchsin, itself known to be a characteristic of ageing. The gradual loss of covalent bonding of the two strands of DNA helix with age indicates a possible manner in which somatic mutations may act. Accumulation of such somatic mutations may exceed in due course the repair mechanism of the DNA, leading to its failure with ageing.

Another genetic mechanism of ageing presented by Martin (GM 1977 In: *Molecular human cytogenetics* ed Sparkes RS *et al.* 7: 355) suggests that ageing is very likely related to an accumulation of defects which can be removed only by recombination and repair between homologous chromosomes. Such defects might arise from incomplete repair and DNA cross linking and senescence may be related to cell division because these defects form primarily during DNA replication. Ageing and rejuvenation in germinal cells may have evolved as a byproduct of mechanisms which promote genetic recombination during synapsis.

Evidences supporting this concept are the association of deficient or abnormal DNA repair with progeria and Cockayne's syndrome; of ageing with the formation of tightly linked DNA-protein complexes, of damage to ribosomal genes in certain senescent tissues and the accumulation of DNA nicks in some cases. Since the process involves the germ line cells, the mechanism must be reversible. Despite these evidences, however, it is difficult to visualise a complete repair of the DNA thread during genetic recombination in which the parental characters are maintained in full.

An evolutionary view of ageing, which supports the *error catastrophe* concept, suggests that mortality may be due to an energy-saving strategy of reduced error regulation in somatic cells (Kirkwood TBL 1977 *Nature* **270**: 301).

Medawar (1952) on the other hand was of the opinion that ageing is the result of natural selection delaying the time of onset of deleterious genes, so that senescence and death result from the accumulation of late-acting harmful genes. This theory presupposes ageing in a general sense since the action of a gene during adulthood is determined by its biochemical environment, rather than its chronological age.

## Theories advocating genetically programmed ageing

The second category of theories regards the loss or suppression of molecular information or synthetic capacity as a necessary step in differentiation and development. It assumes that gene regulation and sequential gene action during developmental processes control age changes as well. That is, age changes, similar to developmental changes, are programmed in the original pool of genetic information and follow the latter in an orderly sequence. An obvious corollary is that prolonging the process of development may retard age changes. Rats, kept on a restricted diet, inspite of an increased metabolism, had a longer lifespan than fully nourished controls, presumably due to retardation in development. Serial gene expression has been recorded in some ciliates, which may have some relevance in this connection. The role of genetic regulation is also indicated by the hormonal control over gene action in *Chironomus* larvae, the transformation of foetal to adult haemoglobin in man and the progressive decrease in activity of certain enzymes with age in different animals or during maturation of the human foetus. The period required for induction of enzymes like tyrosine-amino-transferase in mice, after brief exposure to low temperature, is found to increase with age, although after induction, the rate of production is independent of the age of the animal. These observations indicate the presence of a system of repression and derepression at the genic level, which may control age changes, rather than an alteration of the basic genetic composition through mutations and/or errors. The qualitative changes of enzymes during senescence, often reported (Singh SN and Kanungo MS 1968 *J Biol Chem* **243**: 4526), may, however, be explained through both genetic regulation and somatic mutation theories.

## Evidences from tissue culture

The demonstration of a limited life span of normal mammalian cells in culture opened up new prospects for assessing the different theories of senescence.

Diploid cells of human origin in culture were observed to exhibit a finite proliferative capacity *in vitro* in terms of the number of population doublings (50±10) they can achieve, which leads to eventual degeneration and loss of the culture. A similar phenomenon was recorded in chick, bovine, horse, wallaby, grey kangaroo and marsupial cells in culture. The cultures can be serially subcultivated many times, but eventually growth slows, debris accumulates and the population is lost. This behaviour has been interpreted as a manifestation of senescence at the cellular level. Evidences indicated that the age changes observed during cell culture may be of relevance to those occurring *in vitro*. For example, in cells cultured from human donors of different age groups, an inverse relationship could be recorded between the age of the donor and the lifespan of the culture.

Detailed studies on the diploid cells in culture show that they undergo a regular decline in proliferative activity. The individual cells in the population proceed asynchronously from a rapidly dividing, through a series of more slowly cycling states, to a final state of arrest or apparent arrest (see Cristofalo VJ 1977 in: *Senescence* ed Nichols WW and Murphy DG Plenum New York). These changes are accompanied by several well-defined physiological and biochemical changes.

A study of the three phases of *in vitro* senescence, involving the initial setting up of culture, rapid proliferation following subcultivation and gradual slowing, was utilised in the analysis of cellular ageing. The results were interpreted to support the different hypotheses, ranging from a developmental model of programmed senescence to those implicating random errors in cellular nucleic acid and protein processing machinery.

Based on cytophotometric measurements of DNA contents in human foetal tissues in culture (Mulherkar R, Talukder G and Sharma A 1979 *J Sci Ind Res* **38**: 322) it may be suggested that in mammals at least the ultimate process of senescence is due to the combined effects of intrinsic programming of DNA replication and the accumulation of defects in the macromolecules through cytoplasmic errors (Sharma A and Talukder G 1979 *Nucleus* **22**: 128).

## Conclusions

Most recent theories of ageing postulate, directly or indirectly, a genetical basis. The concepts belonging to the *general error* series suggest senescence to be the outcome of the accumulation of errors in the fidelity of macromolecule synthesis (whether in

DNA, RNA or protein synthesis or in DNA repair). The rate of accumulation of such defects in the macromolecules is itself genetically determined. Therefore, species with differing lifespans will vary in the relative accuracy of the production of such molecules with time. The *genetic programming* theories, on the other hand, regard the process of ageing as an intrinsic part of the developmental programme, ultimately depending on the genotype. The ultimate result may be due to the combined effects of intrinsic programming of DNA replication and the accumulation of defects in the macromolecules through cytoplasmic errors.

CHAPTER IV: 9

# EXTERNAL AGENTS IN THE INDUCTION OF GENE AND CHROMOSOME ALTERATIONS

The effects of outside agencies on chromosomes received progressive attention after the reports of X-ray induced mutations in Drosophila by Müller in 1927 and by Stadler in maize. These discoveries led to the observation of mutagenic properties of chemicals by Oehlkers in 1943 and Auerbach and her colleagues in 1946, followed by a host of others. Avery and Blakeslee utilised the chemical colchicine in inducing polyploidy in plants. Effects resembling those of radiation were referred to as radiomimetic effects and chemicals inducing them as *radiomimetic* chemicals (see Sharma AK and Sharma A 1960 *Int Rev Cytol* **10**: 101).

## Types of chemicals involved

In the past decade, increasing awareness of the importance of environmental factors has led to greater emphasis on prevention and to attempts to minimise human exposure to hazardous chemicals. There are thousands of natural substances to which humans are exposed and, in addition, man-made chemicals have become an integral part of modern society. Approximately 4.5 million chemicals are known to exist and their rate of numerical growth is about 10% per year (Fishbein L 1980 *J Toxicol Env Hlth* **6**: 1133). Quite a large number of them have been identified for their mutagenic, clastogenic, carcinogenic and teratogenic effects. Compounds producing chromosome abnormalities usually also induce gene mutations. Thus the induction of chromosomal alterations may be taken to indicate genetic damage as well (see Sugimura T, Kondo S and Takebe H 1982 ed

*Environmental Mutagens and Carcinogens* University of Tokyo Press and Alan R Liss Inc New York).

The chemicals identified as carcinogens and mutagens represent a wide spectrum with a range of varying biological activities. Some of the common mutagens have been identified among the food additives, hair colourants and various industrial chemicals. Of the food additives, saccharin was reported to be occasionally carcinogenic, leading to its ban in the USA in 1977 in food, drugs and cosmetics. Pesticides are widely used. Some of them, like DDT, have a long term effect and enter the food chain. The effects of DDT and its metabolic products have led to a large amount of information being gathered on the genetical effect of these compounds and ban on some of them in Japan and the USA.

Some drugs have also been observed to induce chromosome damage in man, including daunomycin. Amongst the stimulants, higher incidence of chromosomal aberrations has been reported in lymphocytes cultured from chronic alcoholics, as compared to control. The action of caffeine is more controversial, though a high intake has been observed to lead to a predisposition to loss of reproductive ability in women. In higher doses, nitrophenylinediamines, which are widely used in hair colourants, are in some cases found to be mutagenic or carcinogenic in mice. Mutagen and clastogen testing, therefore, has become an important part of toxicology (see Berg J 1979 ed *Genetic damage in man caused by environmental agents* Academic Press New York).

Since the isolation of a pure carcinogenic chemical benz(a) pyrene in 1932, more than 1500 chemicals with widely different structures, isolated from natural or environmental sources or synthesised, have been shown to be carcinogens and/or mutagens in different test systems. Though no single molecular feature has been pinpointed to be carcinogenic, yet intensive investigations have demonstrated that almost all chemical carcinogens, in their ultimate forms, are highly reactive electrophiles. Direct alkylating agents are electrophilic by nature and most other compounds undergo metabolic conversion to these reactive intermediates. These get covalently bound to tissue nucleophiles in the target organs and may initiate cancer or mutagenesis (see Emmelot P and Krick E 1979 ed *Environmental carcinogenesis*

Elsevier/North Holland Amsterdam).

The electrophilic species generated from xenobiotics *in vivo* can be broadly classified into:

(a) nitrenium ions or nitrenes arising from functional moieties, nitro, nitroso, amino (primary, secondary and tertiary), acetamido, N-hydroxyl amino, etc.

(b) epoxides from compounds, having double bond as polvcyclic aromatic hydrocarbons, aflatoxins and amidopyrin, etc.

(c) carbo-cations from N-nitroso compounds, cycasin, substituted hydrazines, etc. (see Fishbein L 1979 *Potential industrial mutagens and carcinogens* Elsevier Amsterdam).

A number of chemicals are known to induce chromosomal aberrations. They are also known as *clastogens.* In contrast to the X-rays, where such chromosomal changes can be detected almost immediately, the effects of these chemicals are delayed. They act on the $G_1$ and early S phases of the cell cycle and the appearance of the aberrations depends on DNA synthesis. Based on these two criteria, that is, the type of aberrations produced and the stage in the cell cycle affected, chemicals may be divided into four classes:

(i) Chemicals producing gaps (achromatic lesions) and deletions after exposure in late S and $G_2$ phases. The biosynthesis of DNA and DNA precursors is inhibited. Relatively few compounds show this effect, including hydroxyurea, cytosine arabinoside, deoxyadenosine and 5-fluoro-deoxyuridine.

(ii) Chemicals producing all types of chromatid aberrations after exposure in late S and $G_2$ phases. They are also few in number, like 8-ethoxycaffeine and streptonigrin. Earlier workers classified their effects as non-delayed, radiomimetic and independent of S-phase.

(iii) Chemicals producing all types of chromatid aberrations but only in cells treated in $G_1$ and early S. Most chemical clastogens belong to this group, including alkylating agents and nitroso compounds. The effect is delayed and is dependent on S phase for expression of aberrations.

(iv) Compounds which inhibit DNA repairs such as caffeine.

**Test systems**

Standard test systems have been devised to evaluate adverse long-term chronic health effects, such as cancer and heritable mutations, for chemicals of both everyday and rare use. Animal carcinogenicity tests are very expensive, time-consuming (about 3 years) and their sensitivity is limited. In testing for heritable germline mutations, the time required is about 2 years but a very large number of animals is used (up to 50,000) for a relatively low number of potential human mutagens.

These difficulties have led to numerous short term techniques for detecting potential chemical carcinogens and mutagens, involving both prokaryotic and eukaryotic test systems. More than 100 such methods are available (see Hollaender A and De Serres FJ eds 1971-1980 *Chemical Mutagens* **1-6** Plenum New York). Various agencies, like Environmental Protection Agency (EPA), International Commission for Protection Against Environmental Mutagens and Carcinogens (ICPEMC) and World Health Organisation (WHO) are advocating the use of such tests in assessing the carcinogenic and mutagenic potential of chemicals in humans.

Tests with micro-organisms are widely used in screening for mutagens due to the rapid growth rate of the cells, the large number of cells which can be tested and the technical simplicity and low cost of the tests (see Angelosanto FA and Nichols WW 1979 *Mutat Res* **65**:133).

The current most widely used short-term test is the Salmonella/Ames test using several specially constructed strains of *Salmonella typhimurium* in culture for detection of frameshift or base pair substitution reverse mutations in auxotrophs. Rodent (or human) tissue homogenates (S9 mix) are employed to metabolise chemicals to their active mutagenic forms. It can detect most of the known organic chemicals with carcinogenic properties but gives negative reaction in testing for metal carcinogens. *Escherichia coli* and *Bacillus subtilis* have also been utilised in identifying mutations in auxotrophs and growth inhibition tests. *E. coli* K-12 is effective in inducing prophage and nucleic acid mutations. Of the lower eukaryotes, different strains of *Saccharomyces*, *Neurospora* and *Aspergillus* are able to indicate mutations in culture, arising through gene conversion, forward and reverse mutations, mitotic recombination and meiotic non-disjunction.

Established mammalian cell lines with stable karyotypes in culture have been employed to a limited extent in testing for mutagenesis at specific loci. Some common cell lines in use are Syrian hamster embryo, Chinese hamster ovary and human diploid fibroblasts, coupled to an external metabolic activation system. They are complementary to tests using the micro-organisms, but are too expensive and too limited to be used as a single criterion in a screening programme.

Several kinds of short-term tests have been developed to detect the ability of certain chemicals to interact with DNA by measuring how much DNA repair is induced (see chapter on Molecular basis of mutation). Since DNA repair decreases the DNA-damaging and mutagenic effects of chemicals, cells deficient in DNA repair can be more sensitive to lethal effects of chemical mutagens.

Of these, DNA-repair synthesis can be measured *in vitro* using human or rodent cells or *in vivo* in germinal or somatic tissues, through autoradiography following incorporation of radioactive label in repaired DNA. Techniques otherwise used for detecting DNA strand breaks can to some measure be applied to testing for chemical mutagens. These include alkaline elution, alkaline sucrose—gradient sedimentation, DNA binding assays and nicking of superhelical DNA.

The techniques for measuring inhibition of DNA replication are less adequate in detecting mutagenic effects of chemicals on a large scale since the relationship between mutagenesis and inhibition of DNA replication is not yet fully understood. Reports are, however, available of the inhibition of DNA synthesis *in vivo* in testicular tissue of mouse with different chemical agents. This method may be utilised in finding out if a chemical has reached the germinal tissues.

Insects, particularly *Drosophila*, are used widely for sex-linked recessive lethal (SLRL) test and also in screening for dominant lethal translocations and sex chromosome and partial chromosome losses. In the former, treated males are mated with untreated females. A X-chromosome carrying a lethal mutation inherited from the treated male parent, when passed on to the $F_2$ generation by the daughters, leads to lethality of the male offspring.

*In vivo* tests in mammals include both effects on germline and somatic tissues. The main value of such tests is indicating whether the active form of a chemical mutagen can reach the germline to induce relatively non-specific changes. The limitations of these tests lie in the fact that usually one or more tissue is examined for the effect and the metabolic activation enzymes and even DNA repair enzyme vary widely in different tissues. If the primary metabolic activity of a chemical occurs in a tissue other than the one examined, the mutagenic effect may not be detected. Most such tests detect gross chromosomal effects and often point mutations cannot be identified. In addition, as mentioned earlier, the large number of animals and the long period involved are limiting factors. Heritable germline effects may be screened by testing for specific loci, heritable translocations, sex chromosome anomalies and dominant lethals in rodents, since these effects can be seen in the phenotype of progeny, litter size in $F_1$ and embryo loss. Sperm abnormality may be assayed through observing alterations in sperm morphology. The presence of mosaic patches on fur coat, pigmented retinal cells and haemoglobin variants detected by labelling for antibodies, indicates effects on somatic cells. Other tests, involving histocompatibility changes and skeletal abnormalities are less frequently applied.

The importance of tests for identifying chromosomal alterations induced by mutagens stems from the fact that there is a high degree of correlation between gene mutations and chromosomal changes. Almost all chemical and physical agents that produce one type of effects also induce the other, within limitations of dosage and period of treatment. However, a quantitative correlation between the extent of chromosomal damage and mutations induced by a clastogen is often lacking. For example, methylating agents are more effective that ethylating agents in producing chromosome breakage but the reverse holds good for mutations. Thus for effective screening of the harmful action of chemicals, chromosome aberration tests should be used in combination with methods for identifying point mutations. Such cytogenetic test systems are the only methods at present available capable of detecting *in vivo* human genetic damage. This information is of particular importance in view of the report from WHO that one-third of all spontaneous abortions, 5% of all recognised conceptions and one in every 200 livebirths carry a chromosomal abnormality, The different cytogenetic techniques involve the study of somatic or germinal chromosomes *in vivo* or *in vitro* following chronic or acute exposure to chemicals in a variety of species. For scoring chromosomal changes, the systems may include divisional stages with banding patterns to identify the segment involved or differential chromatid staining to detect sister chromatid exchange (SCE) frequency (see Hsu TC 1982 ed *Cytogenetic assays of environmental mutagens*, Allenheld Osmun, Totowa, NJ; and Sharma A 1984 *Environmental Chemical Mutagenesis* Persp Rep Ser 6, Golden Jubilee Publ, Indian National Science Academy, New Delhi).

Amongst the *in vivo* tests, the micronucleus test is based on the observation of extranuclear bodies in the cell cytoplasm arising from lagging of fragmented or whole chromosomes. They are scored in cells that have completed one or more mitotic cycles after exposure. It is a rapid method, principally applicable to nucleated cells or red blood cells, particularly young, polychromatic erythrocytes. The results are suitable for an initial screening. Its application is however not wide since only chromosome breaks or laggards can be scored and not even the nature or type of breaks.

Mammalian *in vivo* studies are usually preferred to *in vitro* ones because the metabolic activation and detoxification of the chemicals in the intact animal are closest to the human system. *In culture* as well, liver microsomal fractions that contain mixed function oxidases may be used, to simulate *in vivo* conditions. Basically the method involves exposure of the animal to acute or chronic doses, imbibed orally or intraperitoneally. Chromosome aberrations are scored from bone marrow, and/or other tissues like thymus, spleen and spermatogonia. Conventionally stained metaphase preparations are adequate for detecting major chromosomal alterations. Banding procedures, on the other hand, detect a higher yield of balanced abnormalities following chronic exposure. Instead of bone marrow, in case of human populations exposed to environmental hazards, short term cultures of peripheral blood can be studied as well. Since the blood lymphocytes circulate throughout the body, they are presumed to have been exposed to the activated products of the chemicals as they pass through the liver or other organs capable of activating or detoxifying them. This method has been used in monitoring humans subjected to chemotherapy or to potentially mutagenic substances, either accidentally or as an occupational hazard. *In vitro* studies for chromosome aberrations allow accurate control of period and dosage of chemical used. Higher doses can be tested than is possible with intact animals. The culture tissues used are usually rodent cell lines or human fibroblast or lymphocyte cultures. This method is quite successful in detecting known carcinogens through the induction of identifiable chromosome aberrations.

Sister chromatid exchanges (SCE) are the result of an exchange of DNA and its replication products and can be identified in the metaphase chromosome through a symmetrical exchange between sister chromatids at a homologous locus. Cells are grown in the presence of BrdUrd, a thymidine analogue, for two replication cycles. In the subsequent metaphase, each chromosome consists of a unifilarly substituted chromatid and a bifilarly substituted one. They can be identified by staining differentially with fluorochromes and Giemsa and the regions exchanged can be clearly detected. Exposure to X-rays and chemicals are seen to increase the frequency of the exchanges, This test can be used both *in vivo* and *in vitro*. Though it gives negative results in some cases as with metal mutagens and some classes of DNA-intercalating agents, yet it can serve as a useful test for mutagens in combination with other methods. (see also chapter on Methodology).

A standard test was devised for the study of chemicals on chromosomes of plants. Initially *Allium cepa* was chosen as the experimental material due to the easy availability of roots, low cost and facility in handling. During the experiment, commonly referred to as *Allium* test, bulbs were placed at the mouths of jars containing the chemical, with the roots immersed. Root-tips were excised after desired periods and chromosome alterations recorded after squashing. Other plants, like *Vicia faba*, barley and *Tradescantia* have also been used to locate specific alterations.

### Biochemical action of mutagens

(see also chapter on molecular basis of mutations)

The analysis of mutagenesis has proceeded with the understanding of gene functioning. The mechanism for inducing mutation may involve either of the two phenomena (i) directly induced base mispairing and (ii) base mis-repair. In the former spontaneous or induced mispairing results in specific base pair substitutions. In the latter indirect method, mutations are one or more steps removed from the initial event. Alkylating agents give rise to many different reaction products in DNA, but only few of them ($0^6$-alkylalanine and $0^4$-alkylthymine) have been found to induce direct mispairing. Heat is an effective mutagen as well, particularly in large genomes. It converts cytosine to uracil and guanine to an analogue of cytosine, leading to base mispair. When the elongation of a DNA chain is interrupted by the formation of lesions through the action of several products of alkylation, the post-replication repair process is often prone to errors, due to the incorrect insertion of bases into gaps in the progeny-strand DNA opposite such a lesion.

Unlike mutagens inducing direct base mispairing, agents that trigger misrepair ultimately produce a wide variety of mutational types, like transitions, transversions, frameshift mutations and deletions.

DNA polymerases are possibly the most important enzymes of DNA synthesis which influence the rate of spontaneous or induced mutations. These enzymes, being responsible for the fidelity of DNA replication may either (i) select the incoming bases on the basis of instructions received from the parental bases or (ii) select the bases first exclusively on the basis of their hydrogen bonding and then correct the misinsertions by "copyediting". Certain metals, known for their binding action with DNA polymerases, are thus found to be mutagenic. Manganese, for example is highly mutagenic and stimulates the misincorporation of both ribose and deoxyribosenucleotides in culture systems.

**EFFECTS ON CELL DIVISION:** Phases of cell division affected by chemicals are: (i) the stage when the cells enter into division; (ii) the initiation of spindle formation; and (iii) cytokinesis.

Since DNA synthesis and oxidative phosphorylation are necessary in cell division, mitosis is usually inhibited by chemicals which affect these processes. For example 5-fluorodeoxyuridine (FudR), deoxyadenosine (AdR), cytosine arabinoside and

### TABLE IV : 9.1

Classification of chromosome breaking chemicals (after Kihlman 1970)

| *Chemical action* / *Cytological effect* | | *Inhibition of desoxyribonucleotide synthesis* | *Denaturation or degradation of DNA* | *Production of abnormal labile DNA by chemical reaction and/or incorporation of abnormal precursors* | *Removal of DNA-bound metals* |
|---|---|---|---|---|---|
| Both nondelayed and delayed effects | Nondelayed effect fragment | Deoxyadenosine<br>Cytosine-arabinoside<br>5-Fluorodeoxyuridine | | | |
| | Nondelayed effect of exchange type | | 8-Ethoxycaffeine<br>1, 3, 7, 9,-Tetramethyl uric acid<br>Actinomycin D<br>Streptonigrin | | |
| Delayed effects only—Chromatid aberrations of exchange type | | | | Alkylating agents (mustards, epoxides, ethylene imines, sulphonic esters, alkyl sulphates, diazoalkanes, alkylnitrosamines, nitrosoalkylurethanes, β-propiolactone, mitomycin C, azaserine, Acridine orange)—visible light<br>5-Bromodeoxyuridine<br>Maleic hydrazide | Potassium cyanide<br>Cupferron<br>αα'—Dipyridyl Dieca<br>8-Hydroxyquinoline<br>Adenine |

aminopterin are capable of inhibiting mitosis due to their property of inhibiting DNA synthesis specifically. When the DNA synthesis is resumed through removal of the block, mitosis starts.

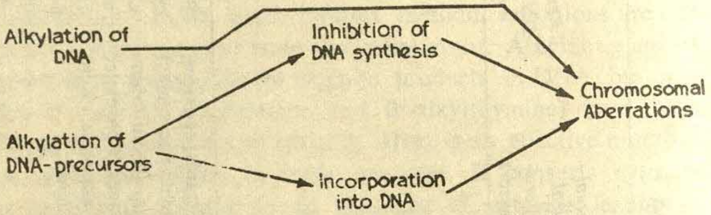

**Fig. IV : 9.1 The possible sequence in production of chromosomal aberrations by alkylating agents (Kihlman B 1971 in *Adv in Cell and Molecular Biology* Academic New York)**

Respiratory inhibitors like carbon monoxide and uncoupling agents, like dinitrophenol can also suppress cell division.

Agents inhibiting the first stage inhibit successively the division of the cell, the nucleus and the chromosomes. The stage affected is interphase and occasionally early prophase.

NATURE OF EFFECTS: The effects on chromosomes may be divided into two categories: *reversible* and *irreversible*. In the former, certain characteristic features are observed: certain vital processes are switched off, but enough metabolic activities are retained which permit the tissue to recover when the external stimulus is removed. Irreversible effects either result in instantaneous death or in toxicity leading ultimately to death of the tissue.

The observed reactions of the different chemicals can be divided into three main zones: lethal, narcotic and subnarcotic.

*Lethal*: Within the lethal zone, different kinds of effects are observed. The reactions are irreversible, leading to ultimate lethality in the tissue. The chemical may act as a fixative and preserve the vital structure, another chemical may give pycnotic clotting of the structure and yet another a swelling up of the nucleus with dispersal of the nuclear matter all over the cell. Amongst the latter is liquefaction of the matrix so that chromatin matter liquefies and gathers at the ends and may even drop off from the chromosomes. Long treatment may result in the thinning of the chromosomes into residual threads. The extraction of nucleic acids by non-fixing chemicals has helped in studying the ultimate chromosomal threads. Heterochromatin is more resistant to the extraction of nucleic acid than euchromatin and the centromeric heterochromatin more so than that in the other regions. With high pH and treatment with potassium cyanide, despiralisation occurs and cells in division swell and die.

*Narcotic*: The main effects observed within the *narcotic* zone are C-mitosis or stathmokinesis and C-tumour formation. C-mitosis, so named because it was first observed with colchicine, takes place through the breakdown of the spindle after the chromatids have separated at the end of metaphase so that they lie within the same cell without subsequent cell plate formation. When the tissue is allowed to recover, the chromosome number is doubled resulting in polyploidy. Prolonged treatment may lead to high degrees of polyploidy as observed with gammexane followed by multipolar stages on recovery. The C-mitotic activity is inversely proportional to its solubility in water in case of most chemicals. Colchicine is, however, an exception. It is highly soluble in water but even very low concentrations (0.5 percent) are capable of causing spindle inhibition and arresting metaphase. As a result a large number of metaphases can be obtained. C-tumour formation results in the formation of bead-like swellings in the root-tips. The cells, due to loss of polarity, undergo disorganised division. This effect may occur independently of C-mitosis though it usually accompanies the latter. Gavauden divided C-mitotic chemicals into two groups—(a) those in which the threshold follows the physical property of the chemical, e.g., solubility, showing that the effect depends on a physical action, and (b) those in which a large margin is observed between reaction threshold and water solubility, indicating involvement of chemical reactions. An example is colchicine, in which, one exchange of methoxy and aldehyde groups in the C rings, forms iso-colchicine. The latter does not have C-mitotic activity.

Metaphase arrest has been observed with several other chemicals, like acenaphthene, chloral hydrate, gammexane, etc. and can be utilised in the analysis of karyotypes.

*Subnarcotic*: Within the *subnarcotic zone*, several effects can be observed. However, the reaction is a reversible one, since the tissue, if removed from the chemical, recovers its normal activity after a certain period.

The different phenomena involved include:

(a) *Pseudochiasmata*: Certain regions of mitotic anaphase chromosomes do not separate, showing bivalent-like configurations. The result is usually fragmentation and ultimate loss of the cell.

(b) *Chromosome erosion:* Parts of the chromosomes are digested, resulting in a "starved' appearance.

(c) *Fragmentation* or chromosome breakage can be caused by a wide range of chemicals. The mode of action is variable, as discussed earlier, though the chromosome component involved is finally DNA. The final upset of the nucleic acid metabolism results in hazards in protein reduplication, causing the chromosomes to break at different loci.

Fragmentation, followed by translocation, may lead to a new pattern of chromosome rearrangements, resulting in heritable phenotypic differences. The chemotherapy of cancer is based on the excessive fragmentation of chromosomes through chemical agents. Several chemicals break chromosomes at specific loci, showing the differential chemical nature of the segments; e.g., EOC at nucleolar organiser and hydroxylamine at primary constriction regions (Fig. IV: 9.2).

(d) *Translocations* are also observed as anaphase bridges.

These reactions usually do not have a threshold within the zone.

Some chemicals show all these three zones, others may show only one or two, depending on the concentration used. In *Drosophila* and maize, compounds related to mustard gas predominantly induce lethal changes, many of which are small deficiencies. The effect is often delayed, leading to mosaics or normal and mutant cells.

Certain organisms possess antimutagens which counteract the mutagenic action of normal metabolic chemicals. For example, catalase checks the action of hydrogen peroxide, which is increased by potassium cyanide. The latter inactivates catalase but normally has a lethal effect. Other chemicals, in combination, may give additive or synergistic effects.

A working hypothesis presented by Kihlman (1971) suggests that DNA is the key substance in chromosome breakage and rejoining and that essentially the same biochemical mechanisms are involved in dark repair of DNA, in genetic recombination and in the formation of chromosomal aberrations.

ASSESSMENT OF MUTAGENIC EFFECT: There are four aspects of study in assessing the mutagenic property of a particular chemical, namely,

(i) What does a substance actually do in somatic and germ cells?

(ii) What proportion of a population is subject to it?

(iii) How much will it enhance the natural mutation rate?

(iv) What will be its consequences on the population as a whole?

The replies can be calculated from the first and second but the fourth one can only be guessed at present. The second problem can be extended to include parameters like age, reproduction and survival. The first aspect can be tested on simple organisms though the results are unreliable when extrapolated to humans. Definite differences are observed in the sensitivity to chemical and physical agents between male and female germ cells. In the interpretation of negative results, statistical significance is required. The cumulative effects of small doses should also be taken into consideration. The increasing rate of pollu-

**TABLE IV : 9.2**

**Current estimate of the human genetic burden[a],[b]**

| *Genetic basis* | *Affected individuals per thousand* |
|---|---|
| Chromosome abnormalities | 6.86 |
| Autosomal dominant gene mutations | 1.85-2.64 |
| Autosomal recessive gene mutations | 2.23-2.54 |
| Sex-linked gene mutations | 0.78-1.99 |
| TOTAL | 11.72-14.03 |

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[a] An additional 10-30% of congenital malformations and other complex disorders are believed to be the result of genetic alterations.

[b] From Committee on Mutagenicity of Chemicals in Food Consumer Products and the Environment, British Department of Health and Social Security Guidelines for Testing of Chemicals for Mutagenicity, 1979.

**TABLE IV : 9.3**

**Techniques proposed for environmental and human monitoring**

| *Environmental monitoring* | *Individual human monitoring*[a] | |
|---|---|---|
| | *Sample source* | *Technique* |
| Work/environmental sample collection subjected to predetermined *in vivo*, *in vitro*, and submammalian assays<br>Particulate collection | Blood | Chromosome aberrations<br>Micronuclei<br>SCE<br>Alkylated haemoglobin |
| Condensation samples<br>Solid/liquid process materials | Urine | Assessment of mutagens excreted via assays using non-human target cells |
| Samples can be evaluated directly or following concentration, fractionation or extraction procedures | Faeces | Assessment of mutagens excreted via assays using non-human target cells |
| Upper limits of release can be set on the basis of available control technology.<br>Levels which exceed these limits can trigger more intense monitoring on an individual basis. | Sperm (semen)[b] | Spermhead abnormalities<br>Fluorescent Y body technique |

[a] Results must be corrected for effects not related to occupation.

[b] These techniques are strictly limited to males and thus are unsatisfactory for general population screening.

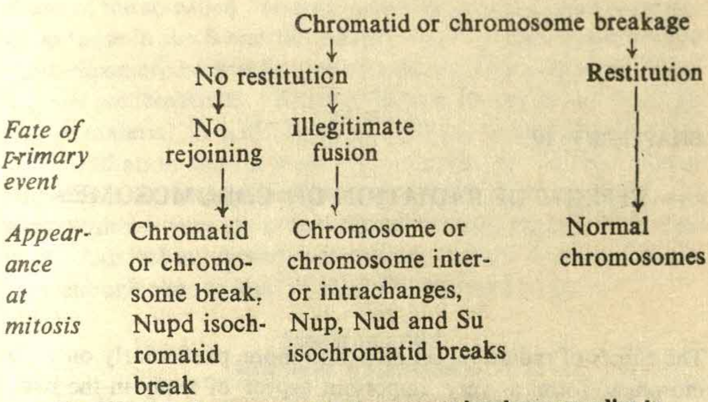
Chromatid or chromosome breakage
No restitution
Restitution
Fate of primary event
No rejoining
Illegitimate fusion
Appearance at mitosis
Chromatid or chromosome break, Nupd isochromatid break
Chromosome or chromosome inter- or intrachanges, Nup, Nud and Su isochromatid breaks
Normal chromosomes

**Fig. IV : 9.2 Consequences of chromosome breakage according to breakage-rejoining hypothesis**

tion in the atmosphere and the progressive use of chemicals in different facets of everyday life have made the study of the genetic effects of chemicals essential before release (Tables IV: 9.2 and 9.3).

## CHAPTER IV: 10

# EFFECTS OF RADIATION ON CHROMOSOMES

The effects of radiation on cells and more particularly on chromosomes, form a very important aspect of study in the background of radioactive fallout and of x-ray therapy. After the first effect of irradiation observed by Müller in 1927 in inducing mutants of *Drosophila* through x-rays, this subject has assumed immense proportions.

Radiations may be ionising or non-ionising. Ionising radiations include x-rays, α, β and γ-rays, fast neutrons and the radiation emitted by radioactive substances. They act by the conversion of the molecules of the material through which they pass into electrically charged ions. They are then converted into electrically charged free radicals, like $H^+ + OH^-$ from water, which are capable of ionising further molecules. The intensity of an ionising radiation is measured by the number of ionisations induced per unit matter, such as 1 *roentgen* being the intensity required to induce one million million ionisations per 1 ml of matter. Compared to the total mass of a living organism, the dosage required for lethality is rather low, particularly in mammalian cells. Non-ionising radiations, like UV rays and infrared rays, cause dissipation of the energy by molecular excitation within the tissue, the principal biological action being attributed to the differential absorption of energy by the cellular constituents, particularly nucleic acids.

The primary changes induced by x-rays include structural alterations of chromosomes following breakage and rejoining. The secondary effects arise from crossing between a normal and a primarily altered chromosomes. Chromosomes at dividing stages are more susceptible to such changes, the meiotic chromosomes being more sensitive than mitotic ones. Breaks in the $G_1$

phase of the so-called "resting" stage lead to chromosome breaks while those in the S and $G_2$ phases result in chromatid breaks. Most chromosome breaks are restituted, only a minority forming new combinations. Restitution may be prevented partially if the material is centrifuged or exposed to supersonic waves after irradiation since it leads to a number of "wrong" recombinations and further structural aberrations. The same dosage given within a very short time shows a larger number of aberrations than when spread over a longer period since the breaks have enough time to heal (Tables IV: 10.1 and 10.2).

**TABLE IV : 10.1**
**Definitions of aberrations**

| *Term* | *Definition* |
|---|---|
| Chromatid gap | An achromatic region in one chromatid, the size of which is equal to or smaller than the width of the chromatid |
| Chromatid break | An achromatic region in one chromatid larger than the width of the chromatid. It may either be aligned or unaligned |
| Chromosome gap | Same as (i) only in both chromatids. |
| Chromosome break | Same as (ii) only in both chromatids |
| Chromatid deletion | Deleted material at the end of one chromatid |
| Fragment | A single chromatid without an evident centromere |
| Acentric fragment | Two aligned (parallel) chromatids without an evident centromere |
| Translocation | Obvious transfer of material between two or more chromosomes |
| Triradial | An abnormal arrangement of paired chromatids resulting in a triarmed configuration |
| Quadriradial | An abnormal arrangement of paired chromatids resulting in a four-armed configuration |
| Pulverised chromosome | A spread containing one fragmented or pulverised chromosome |
| Pulverised chromosomes | A spread containing two or more fragmented or pulverised chromosomes, but with some intact chromosomes still remaining |
| Pulverised cell | A cell in which all the chromosomes are totally fragmented |
| Complex rearrangement | An abnormal translocation figure which |

(*TABLE IV: 10.1*) *Contd.*

| *Term* | *Definition* |
|---|---|
| | involves many chromosomes and is the result of several breaks and mispaired chromatids |
| Ring | A chromosome which is a result of telomeric deletions at both ends of the chromosome and the subsequent joining of the ends of the two chromosome arms |
| Minute (min) | A small chromosome which contains a centromere and does not belong to the karyotype |
| Polyploid or endoreduplication | A cell in which the chromosome number is an even multiple of the haploid number ($n$) and is greater than $2n$ |
| Hyperdiploid | A cell in which the chromosome number is greater than $2n + 1$ but is not an even multiple of $n$ |
| Dicentric | A chromosome containing two centromeres |

The principal factors affecting radiosensitivity include both internal ones like genotype, chromosome type, size and number, stage of cell division, level of ploidy and age of the tissue and external ones like moisture, temperature, presence of oxygen and other atmospheric gases, dosage, storage, presence of other chemicals and ionisation density of the rays used.

The two theories regarding the mode of action of x-rays on chromosomes are the direct hit or target theory and the chemical theory.

The *target theory*, first postulated by Lea, suggests that a direct hit by an electron may cause a gene to mutate, bringing about a chemical change with a different phenotypic expression or a chromosome region to break. It was originally supported by a linear relationship between the x-ray dosage and degree of gene mutation on the basis that a larger number of electrons would cause a larger number of hits. However, this relationship has not been observed in lower dosages and different species behave differently even with the same dosage.

The alternative *chemical theory*, given by Koller and his colleagues, holds that the x-rays possibly ionise the water in the cytoplasm, forming $HO_2^{--}$and other precursors of $H_2O_2$ which

TABLE IV: 10.2

X-ray effects on different stages of a typical mammalian cell[a]

| *Stage :* | $G_1$ | $S$ | $G_2$ | $M$ |
|---|---|---|---|---|
| Events : | $GAP_1$ | Synthesis of DNA | $GAP_2$ | Mitosis |
| Relative DNA content : | 1 | 1—2 | 2 | 2—1 |
| Common types of aberrations observed[b] | Chromosome breaks<br>Rings<br>Translocations<br>Deletions<br>Dicentrics<br>Fragments | Mixed | Chromatid breaks<br>Quadriradials<br>Triradials<br>Fragments | |
| Relative sensitivity to genotoxic effects : | Low | High | Moderate | Low |

[a] Shown as relative lengths of the total cell cycle period for *in vitro* conditions.

[b] These aberrations are the types observed when the exposed $G_1$, S, or $G_2$ cells are scored at the first metaphase (M1). If the cells are scored at M2 or M3 the aberration types are mixed and lose identity with the cell stage.

are highly unstable oxidising chemicals and act later on chromosomes by oxidation. It was validated by the demonstration that on transplanting a normal nucleus into an irradiated enucleated cytoplasm, breaks are induced in the chromosomes showing that the x-ray effect was mediated by the cytoplasm. Similarly unirradiated bacteria, placed in an irradiated medium, show an increased frequency of mutations. An additional evidence is the production of similar radiomimetic effects by both chemicals and x-rays.

The effect of x-rays is the breakage of the $-SH$ group of protein, accompanied by the breakage of the DNA molecule mainly through the $H^+$ and $OH^-$ ions and involving successively the removal of $-NH_2$ and $-OH$ groups from the bases; oxidation specially of the pyrimidine bases so that even the ring is destroyed in some cases; and oxidation of the sugar, due to which the phosphate-sugar link is broken, resulting in a breakage of the nucleotide.

Some mutagens are found to be able to alkylate $NH_2OH$ and phosphate groups. The breakage of DNA is due to the destruction of the H bonds between $-NH_2$ and OH groups, which give DNA its rigidity, by deamination and alkylation of $NH_2$. In the double helix model of DNA even a very small dosage may cause dealkylation and deamination at one point, duplicated in successive replications from the damaged nucleotide—thus explaining the large effects of even small doses.

On treating the tissue with certain substances like cysteine or cysteamine before irradiation the lethal dose is increased, showing that these chemicals give protection against x-ray damage. Radiation damage is much reduced in the absence of $O_2$, possibly because it interferes with radical formation. Therefore chemicals which act as oxygen acceptors or prevent formation of $H_2O_2$ or dissociation of $H_2O$ may also act as protectors. Others like carboxyl chloride and thiourea may protect the chromatid against direct hit by substituting SH groups instead of proteins.

Harmful effects of x-rays may be direct on the individuals concerned—like radiation burns and later leukaemia, lung, tongue and bone cancer or indirect by affecting the germline resulting in sterility and defective children. The effect is cumulative and radiation sources may be natural atomic fallout, artificial therapy or cosmic rays.

Beneficial effects include gene mutations and structural alterations leading to better strains in agri- and horticulture and cancer therapy.

Investigations of factors governing radiosensitivity help in understanding the mechanism of the effect of ionising radiations, and in getting information used for radiation protection and radiotherapy and in inducing genetic variability in crop plants. Radiation can be used effectively in plant breeding if the frequency of induced mutations can be increased with decrease in chromosomal aberrations, physiological injury and sterility and be directed or controlled.

## SECTION V
# KARYOTYPE AND ITS EVOLUTION

CHAPTER V:1

# KARYOTYPE CONCEPT

CHROMOSOMAL differences reflect in general differences in the genic contents of the individuals. The major variations which can be observed from a comparison of related species can be divided into: (i) variations in absolute chromosome size; (ii) variations in staining properties; (iii) variations in chromosome morphology; (iv) variations in relative chromosome size; and (v) variations in chromosome number, including aneuploidy, euploidy and heteroploidy.

The variations in size may reflect either differences in the number of gene products or proteins produced by the individual, or duplications of genes which influence their interactions and the rate of synthesis of individual proteins. Variations in staining properties are usually indices of differences in the timing of gene action. Changes in chromosome morphology show alterations in gene arrangement which influence their subsequent segregation and recombination. Alterations in chromosome number involve either differences in gene arrangement or genic duplication or deficiency or both. Chromosomal differences thus reflect differences in the source or genic variation while morphological, physiological and biochemical differences indicate variations in the products of gene action as modified by environmental factors.

The Russian school of cytologists, headed by S. Navashin, developed the fundamentals of the karyotype concept from their observations that most species of living organisms show a distinct and constant individuality of their somatic chromosomes and that closely related species have more similar chromosomes than those of more distantly related ones. The karyotype was first defined in 1926 by Delaunay as a group of species resembling each other in the morphology and number of their chromosomes. Since the Liliaceous genera on which he worked (like *Ornithogalum*) are rather homogeneous, the term appeared to coincide with the conception of a genus. However Levitsky (1924, 1931), on the basis of records indicating that the evolution of karyotype in many genera takes place through a series of alterations in chromosome morphology, gave a new definition for karyotype. According to him, karyotype is the phenotypic appearance of the somatic chromosomes, in contrast to their genotype. Amongst plants, their appearance is usually recorded from metaphase stages in the somatic meristems. In certain groups, like the Bryophytes and also in some favourable materials like Liliales, karyotypes can be studied from the gametophytes, the pollen grain for example.

In comparing the karyotypes of different species, six different criteria are usually taken into account, namely, (i) variation in absolute chromosome size; (ii) variation in the position of the centromere; (iii) variation in relative chromosome size; (iv) variation in basic number; (v) variation in number and position of satellites, and (vi) variation in the degree and distribution of heterochromatic regions and repeated DNA segments.

Evolution from taxon to taxon has almost always involved one or more of these characteristics. Their primitive or advanced nature has been judged on the basis of their occurrence in species whose taxonomic positions and relative antiquity are well established through other parameters. All these changes, except that involving absolute size, reflect changes in the chromosome structure and number.

In tracing the role of chromosome changes in evolution, attempts have been made to understand the relevance of chromosome organization to gene action and the control of development and therefore, to understand the nature and disposition of nucleotide sequences and their effects. These experiments are based on the techniques of DNA denaturation and reassociation. The complementary strands of the double helix are separated, cut into shorter strands and permitted to reassociate. The rate of reassociation ($C_0t$ value) gives the frequency of similar nucleotide sequences, since it is proportional to the concentration of homologous sequences. The prokaryotes, including *E. coli*, have unique or single copy sequences which are represented only once in the genome. The eukaryotes on the other hand, possess varying numbers of sequences, which are moderately or highly repeated being represented at ranges between 200 and 200,000 times or more. From relative lengths of sequences of the various categories, DNA content of a genome can be classified precisely. The proportions of repeated DNA sequences may form between 45 and 95% of the entire genome in different plant groups. For example, 12 to 20% of the wheat genome consists of six copies of repeated sequences for hexaploid cell, supporting the uninemic concept of the DNA double helix. Most of the non-repeated sequences are about 1000 nucleotides long and are interspersed between repeated sequences. In most other organisms examined, non-repeated sequences between 800 and 2500 nucleotides in length are interspersed between shorter segments about 300 nucleotides in size. Since the latter approximate in length a protein-coding sequence, it has been suggested that repeated sequences are involved in the control of gene function.

As discussed in the chapter on *repeated DNA*, in some animals, at least some sequences have inverted complementary sequences as neighbours. These are known as *palindromic sequences*. They show very rapid renaturation when the two halves of the palindrome pair together rather than with the other strand of the double helix. *Constitutive* heterochromatin has also been seen to be associated with a high concentration of tandemly arranged highly repetitive sequences not regularly interspersed with other sequences.

These new approaches have led to a modification of the concept of the chromosome evolution in different groups to a broader basis, involving different aspects of structure and function as well as chromosome morphology. The classical study of chromosomal phenotype during meiosis and mitosis has been replaced by banding patterns to reveal greater linear differentiation and DNA measurements giving the relative amounts of repeated and unique sequences.

## I. KARYOTYPE EVOLUTION IN THE PLANT KINGDOM

### Variations in absolute chromosome size

Variations in absolute chromosome size are apparent between different groups as a whole but also, in some cases between different members of the same group. The total mass of chromosomes in a nucleus has been found to be closely related to its DNA content. Apparently at any particular stage of development the ratio of DNA to proteins is relatively constant in eukaryotes and indicates the amount of genic material present.

The distribution of these values usually follows certain regular patterns. The initial regularity is an increase in the DNA content along the evolutionary scale from the lower groups to the angiosperms within the plant kingdom. Within each group, there is a great deal of variation. Sometimes the range of variation within classes, orders or even families may be far greater than that between the classes. A factor responsible may be polyploidy or increase in the basic number. Nevertheless, often genera within the same family show great differences in chromosome size, examples being the two members of the family Leguminosae *Glycine max* and *Vicia faba*, the DNA content of the latter being nearly six times that of the former. In spite of such variations between smaller units of classification, mean and modal values for DNA content in different groups of vascular plants are rather similar.

**REGULARITIES:** Certain regularities recorded include:

(i) The heterosporous taxa, amongst the spore-bearing vascular plants (like *Selaginella* and members of Marsileaceae), tend to have smaller chromosomes than the homosporous ones.

(ii) Amongst the major groups of plants, gymnosperms usually have the largest mean and modal sizes of chromosomes. Certain angiosperms, like *Paeonia*, *Lilium*, *Tradescantia* and the mistletoe genus *Phoradendron*, may have even larger chromosomes.

(iii) Chromosomes of woody angiosperms are usually small with little size difference between related species and genera. Exceptions include the family Annonaceae.

(iv) Chromosomes of herbaceous angiosperms show great size difference between different genera of the same family, sometimes even between different species of the same genus, e.g., *Oxalis*.

**HYPOTHESES:** Several hypotheses have been proposed to explain these differences in size:

(i) A higher amount of DNA in the nucleus is regarded to indicate a higher number of significant genes, leading to greater diversity of gene function. According to this hypothesis, evolution from simple to complex forms has taken place by the addition of functional genes. Evidences from homologous gene sequences in related proteins show that this hypothesis can explain satisfactorily the evolutionary origin of new enzymatic functions. This theory, however, cannot explain the wide differences in DNA content observed between species of the same genus, as in *Oxalis*, or the fact that the chromosome volume per nucleus is higher in the more primitive seed plants, like the Cycads, than in the majority of the highly advanced angiosperms.

(ii) Another theory postulates that the species with larger chromosomes possess larger quantities of DNA with "nonsense" sequences of nucleotides, having no adaptive value. A consequence of this view would be that in the older taxa, species with an initially large amount of DNA would gradually lose it through random deletions. Thus individuals within the same species and related species separated geographically would show variable chromosome size. However, this effect is not seen in actual cases. For example, all diploid species of *Trillium*—a genus existing for 40 to 50 million years—have 5 pairs of very large chromosomes. Though the species are scattered over the temperate areas of the world, yet their chromosomes resemble each other in gross morphology and size. It is obvious therefore that the large amount of DNA present in these chromosomes is not useless and has some adaptive significance.

(iii) The third suggestion is that species with large quantities of DNA have multistranded chromosomes and are therefore genetically equivalent to polyploids, though the somatic number may be diploid one. Comparative measurements of DNA in Droseraceae and Ranunculaceae, indicate that polynemy is responsible for increase in chromosome size during evolution.

(iv) Another hypothesis holds that the presence of large chromosomes indicates the occurrence of gene loci which are replicated many times in series, tandem fashion. Such duplications are regarded as adaptive. Rees H and his group, from studies on *Lolium*, *Lathyrus* and *Allium*, suggest that increase in chromosome size is due, at least in part, to the lengthwise replication of chromosome segments. From a comparison of breeding systems and morphology of phenotypes, chromosome size has been found to increase in *Lolium* and *Allium* and to decrease in *Lathyrus*. Such replication of chromosome segments has been regarded to give opportunities for the differentiation of genes with new function and the establishment of lateral heterozygosity.

The denaturation-renaturation technique followed in the study of mammalian chromosomes indicates the presence of "repetitive DNA", where duplicated nucleotide sequences are present along the DNA thread. In calf liver, for example, about 40% of the DNA contains sequences which are repeated at least 10,000 times (see Section II).

The tandem duplication hypothesis does not refute the multiple strand hypothesis, rather it complements the latter. Both forms of duplication may have been effective in increasing the chromosome size during evolution. The duplication of gene sequences can account for the reverse trend of decrease in size as well. In certain highly specialised forms, decrease in chromosome size occasionally takes place, as for example in *Crepis* and *Muscari*. This trend can be explained in *Crepis* (family Compositeae) by assuming that the evolution of annual growth cycles reduces the adaptive value of extra duplications of certain gene loci, resulting in their elimination. Similarly, in *Muscari* (Liliaceae), the evolution of reduced inflorescences may be associated with smaller chromosomes, occurring from the loss of gene loci rendered useless.

FREQUENCY: Phylogenetic reduction and phylogenetic increase in chromosome size have been found to be about equally common in higher plants and the processes are reversible. In certain groups there is no correlation between geographical distribution and chromosome size. In others, however, species with large chromosomes occupy cooler climates than those with smaller ones.

### Variations in basic number

Alterations in the basic series may or may not be accompanied by polyploidy. In higher organisms they are usually the end product of a series of changes in the $x$ or basic number, followed by different levels of polyploidy. Aneuploids are more frequent than direct euploids or amphidiploids.

The most common alterations in chromosome number in higher plants are duplication or higher multiplication of the entire chromosome complement. In several plant genera the species form a polyploid series, for example, in *Dioscorea*, the species are multiples of $x=10$, from $2n=20$, 40 to 120. Usually the lower numbers are more primitive than the higher ones as seen from taxonomic characteristics. This trend is an irreversible one. However, occasional reduction from higher to lower ploids may also take place. *Euploids* are polyploids which are entirely multiples of a single basic number.

In many cases, multiple series of chromosome numbers are present in species which have arisen by doubling of chromosomes from hybrids between diploid species having different basic numbers. For example, amongst the cultivated species of *Brassica*, *B. napus* shows $2n=38$ in its body cells. The number has arisen by the duplication of the entire chromosome complement of a hybrid between *B. campestris* ($2n=20$) and *B. oleracea* ($2n=18$).

Aneuploid variations form a series in which the gametic numbers of related species are present as a consecutive series or differ from each other by two or more chromosomes. For example, the species of *Crepis* form a series of $x=3$, 4, 5, 6 and 7 chromosomes.

The evolution may involve a descending series as in *Crepis*, where $x=7$ is present in the most primitive species and successively advanced ones have progressively lower basic numbers. Such alterations usually occur through successive unequal translocations which also progressively increase the differences in relative size between the chromosomes. When the smallest chromosomes have heterochromatic regions near the centromere, where the genes show relative inertness, all the essential genetic material can be removed from such chromosomes through a final unequal translocation. As a result, such a truncated chromosome, with only a centromere and adjacent heterochromatic regions, can be easily lost, leading to a reduction in the basic number of chromosomes. This method for decrease in basic number is referred to as *dislocation hypothesis*.

Increase in basic chromosome number has a more elaborate mechanism. The chromosomal rearrangements involved can be studied from organisms subjected to radiation or other chromosome breaking agents, where structural heterozygosity has arisen through reciprocal translocations.

A possible method is by non-disjunction of a small bivalent during first meiotic metaphase, so that one gamete will contain both chromosomes. An offspring of this gamete with a normal one will be a plant trisomic for the small chromosome. Usually such individuals are unstable. However they may survive in certain cases, particularly in interracial or interspecific crosses for indefinite periods. Reciprocal translocation may occur between such a small chromosome and a larger one, so that essential genetical material is transferred to the former. In such cases, the new chromosome will become an essential member of the karyotype leading to an increase in the number.

Occasionally, in artificially induced translocations, a structural heterozygote may have a small fragment with a centromere and attached chromosomal material, derived from one of the normal chromosomes. If another translocation transfers some more essential material to such a chromosome, it will persist, causing an addition to the number in the basic set.

Another type of aneuploidy which is similar to a progressive increase in chromosome number may arise by a progressive decrease or increase in the basic number followed or accompanied by amphidiploidy. For example, in *Crepis* with $x=7$, 6 and 5, different polyploid and amphidiploid combinations can produce every haploid number from $x=10$ upwards. Polyploids of $x=5$ will give rise to $x=10$; $x=11$ can arise from amphidiploids of hybrids between $x=5$ and 6; $x=12$ from $x=6+6$ or $7+5$, $x=13$ from $7+6$, and so on (Fig. V: 1.1). A very extensive aneuploid series is the one observed in the genus *Carex* with haploid numbers from $n=6$ to $n=56$, which have possibly arisen by structural changes of chromosomes followed by autopolyploidy or amphidiploidy. However, this genus, and others of the family Cyperaceae, are characterised by the presence of diffuse centromere, where the property of spindle attachment is distributed throughout the length of the chromosome. All fragments produced in such chromosome complements have a chance of survival, giving rise to various numbers. Translocations, hybridisation between species with different numbers and subsequent chromosome doubling can result in the extensive aneuploid and heteroploid series observed in these genera.

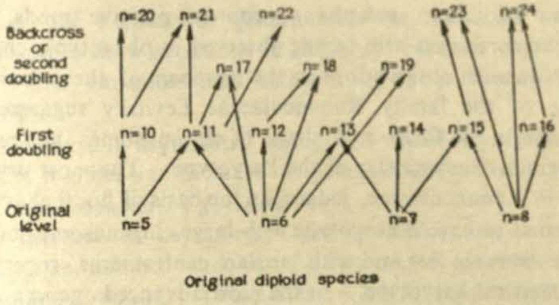

**Fig. V : 1.1 The various methods through which successive hybridisation and chromosome doublings can lead to different aneuploid chromosome numbers (after Stebbins GL Jr 1971 Chromosome evolution in higher plants Edward Arnold London)**

Thus the naturally occurring aneuploid series of chromosome numbers in sexually reproducing plants can be classified into: (i) descending basic, e.g., *Crepis*; (ii) ascending basic, e.g., *Allium*; (iii) interchange polyploid—amphidiploid, e.g., *Brassica*, *Stipa*, *Carex*; and (iv) unbalanced numbers after polyploidy and subsequent apomixis.

In the short-term evolution of plants both auto- and allopolyploidy may have been dominant factors since polyploidy increases the 'biochemical versatility' at the primary enzyme level. It can create new 'hybrid' enzymes through oligomerisation of related polypeptides produced by related (homoeologous) genes. Such enzymes, with different properties, may extend the range of environments in which normal and successful development may occur.

*Crytopolyploidy* refers to an increase in chromosome size, as shown by a series of doublings of minimum genome size, indicated by DNA/genome ratio, rather than an increase in ploidy level.

**Variations in form and relative size of chromosomes** include variations in chromosome segments and heterochromatin as well.

SYMMETRY: Variations in the size and shape of chromosomes have been observed, to a greater or a lesser degree, between species of the same genus and between genera belonging to the same family, having the same chromosome number. In a large number of cases, such changes showed positive trends, which could be correlated with trends observed in phenotypic changes. Based on such observations in the members of the tribe Helleboreae of the family Ranunculaceae, Levitsky suggested that evolution in the form and shape of chromosomes has resulted in progressive asymmetry of the karyotype. The most primitive genus in Ranunculaceae, judged on the basis of floral characters, was found to have a karyotype with large chromosomes of more or less the same size and with median centromeres, regarded as a symmetrical karyotype. In the most advanced genera of the group, *Aconitum* and *Delphinium,* the more advanced species show progressive asymmetry in the karyotype. Therefore, it was suggested that two trends can be observed in the progressive evolution from a "symmetrical" karyotype to an "asymmetrical" one: (i) Reduction in length of one arm of the chromosome, giving rise to submedian and subterminal centromeres from median ones; and (ii) Reduction in size of some chromosomes in relation to others of the same set, so that the advanced karyotype has chromosomes of progressively unequal sizes.

Thus a symmetrical karyotype will have chromosomes of more or less similar size with the chromosome arms of almost equal length. An asymmetrical karyotype has, on the other hand, many chromosomes with subterminal centromeres or greater size difference between different chromosomes or both.

Karyotypes of the different species of a genus may either be alike or may vary greatly between species. Even in those genera where the karyotypes of different species are very similar, like *Paeonia* and *Lilium*, meiotic studies of $F_1$ interspecific hybrids indicate that they differ from each other in inversions and translocations. These differences could not be identified in the karyotypes through conventional methods, because the inversions are paracentric ones and the translocations involve small segments.

In some species the morphological changes may involve alterations in the number of chromosomes with secondary constrictions, as in *Lilium* with progressive polyploidy. Alterations in the patterns of distribution of heterochromatin have been found to accompany speciation in north American *Trillium.*

## Trends in evolution

Most of the herbaceous genera with medium-sized to large chromosomes show variations in karyotype between the species. Usually the variations may follow either of the four following patterns:

(i) Increase in asymmetry associated with decrease in chromosome number and increase in specialisation of certain morphological characters, e.g. *Crepis.*

(ii) Increase in asymmetry accompanied by an increase in chromosome number and certain specialised characters, e.g. *Clarkia* and *Brodiaea.* This pattern is much less frequent than the first one.

(iii) Occurrence of different basic chromosome numbers with little or no change in the symmetry of the karyotype, e.g. *Ranunculus* and *Allium.*

(iv) Constancy in basic number accompanied by variation between species in karyotype symmetry, e.g., *Aegilops.*

There is a predominant trend in flowering plants towards increasing asymmetry of the karyotype, usually associated with greater specialisation in different aspects. This trend can be observed clearly on comparing the more advanced genera like *Aconitum* and *Delphinium* with highly specialised flowers with the more primitive ones like *Nigella* in the tribe Helleboreae.

The morphological effects of structural changes on the karyotype depend on the location of breaks and reunions. Since symmetrical changes need more precise location, obviously asymmetrical changes will be more frequent, leading to progressive asymmetry of the group.

The process of centric "fission", or the breakup of a metacentric chromosome into two telocentric chromosomes, assists in progressive asymmetry. However, the opposite process, that of "centric fusion" between two telocentric or reciprocal translocation between two acrocentrics may correspondingly result in increasing symmetry of individual chromosomes. The former process would give rise to a metacentric and the latter to two metacentrics, one large and one small. Survival of both the new chromosomes would result in increasing asymmetry amongst the members of the karyotype, forming *bimodality*. If the small product is lost, as in *Crepis*, the basic number is reduced, as also the bimodality. If further evolution takes place through subsequent polyploidy and structural alterations, it becomes very difficult to trace the evolution and interrelationships of such taxa. For example, in the Cycadaceae and Podocarpaceae, where differing frequencies of stable telocentrics are found, opinions are divided as to whether evolution has been through centric fission or *vice versa*.

A method for identifying secondary trends towards decreasing asymmetry from primary ones towards increase would be by studying the process involved. Increased asymmetry usually results from pericentric inversions and unequal translocations of chromosome segments, without changing the number of centromeres or chromosomes. The change involved is that of the fundamental number (F.N. or N.F.) of the large chromosome arms. Decreasing asymmetry by centric "fusion" on the other hand leads to a reduction in the number of chromosomes though the F.N. of chromosome arms remains unchanged.

## Adaptive significance

Two hypotheses have been advanced to explain the adaptive significance of karyotype asymmetry during evolution:

(i) The *ease of separation* theory is based on cell mechanics. It suggests that the small or the telocentric chromosomes are more likely to separate earlier than the large metacentrics during prometaphase and metaphase and thereby have a greater chance of survival. Also in the small chromosomes, the heterochromatic regions flanking the centromere will be smaller and thus capable of separating more quickly than those of larger chromosomes.

The fallacies of this hypothesis lie in the fact that since prometaphase and metaphase are relatively short phases, they are unlikely to affect the entire divisional cycle. In addition, if this concept is accepted, there should be a tendency to eliminate all large metacentrics. However, highly evolved genera like *Lilium* and *Delphinium* show two large metacentrics in their karyotypes. Therefore the concept appears to be untenable in view of the existing data.

(ii) The *linked gene cluster hypothesis* visualised the presence of linked clusters of genes on the longer arms of chromosomes within an asymmetrical karyotype. Beneficial genes continue to be added to them by inversion and translocation, while the arms without such clusters would continue to lose genes through deletion and become progressively shorter.

### ROLE OF DNA IN PLANT EVOLUTION

The advent of improved techniques for the measurement to DNA values *in situ* has led to the accumulation of extensive data on the wide variation in DNA content of nuclei and chromosomes in plants. In general, DNA can be categorised into (a) nucleotypic DNA, controlling the parameters like cell volume and size, nuclear volume, chromosome size and divisional cycles; (b) genotypic DNA, including all structural genes coding for enzymes and (c) repetitive DNA. In general haploid (*n*) DNA content per cell ranges over eight orders of magnitude from $1.3 \times 10^3$ to $6.3 \times 10^{11}$ nucleotides. Within certain groups, like algae, gymnosperms and angiosperms, considerable variation occurs in (*2n*) DNA content per cell. A comparison of taxa on the basis of their minimal DNA contents has given a very general positive correlation between DNA content and the degree of evolutionary advancement.

However, ecological factors appear to be related to the DNA content to a certain extent. For example, species of *Pinus* and *Picea* growing in the northern latitudes contain more DNA per cell than those from southern latitudes. Annuals have a lower DNA content than perennials. Ephemeral annual plants contain lower DNA than non-ephemeral ones, while obligate perennials have a higher DNA content than facultative ones. These observations could be related to the rate of development or generation time of the plant. Annuals with a short lifespan have a short period to complete the replication of DNA. Since they require a higher rate of mitosis and a lower rate of meiosis, their DNA content is low.

Seventy % of the DNA in plants is repetitive in nature. Most conifers differ from each other in the amount of repetitive DNA. In gymnosperms, the chromosome number remains the same but the intrachromosomal repetitive DNA changes to a large extent, leading to speciation. Such *incipient* species gradually become genetically isolated through the acquisition of repetitive DNA which may inhibit crossing over.

Some annuals are seen to reduce DNA content under adverse environmental conditions. The amount of DNA also does not appear to be related to the evolutionary status of a group. Some highly evolved species of Gramineae have a low DNA content while the highly evolved orchids have a high one. Therefore, it appears that the decrease or increase of DNA during evolution depends on a harmonious interaction between genotypic, nucleotypic and repetitive DNA. The former two need to act coordinately for the successful evolution of a species while repetitive DNA possibly has an adaptive significance and a role in the isolation of incipient species.

In invading a pioneering habitat, a species can achieve dominance *either* by acquiring an asymmetrical karyotype with diploidy and cross-fertilisation to grow new forms *or* by self-fertilisation to hold together the clustered genes, followed by polyploidy which confers adaptability and/or apomixis to retain the desirable characters.

The extent to which chromosome characters can be regarded to indicate independently the direction of evolution of a group has been questioned by Jones (1970). He has advocated a synthetic approach, viewing the chromosomes in conjunction with all other characters of the organisms in determining the pattern of evolution. Stebbins (1971), however, suggests that the question be resolved on the basis of the relative frequency of certain phenomena observed during the process, trends towards asymmetry being more common than their reversal.

CHAPTER V: 2

# KARYOTYPE EVOLUTION IN THE ANIMAL KINGDOM*

POLYPLOIDY: The haploid numbers of most species of animals lie between 6 and 20, those above and below being comparatively rare. The role of polyploidy in their evolution has been much less significant than amongst the plants. The prevalence of polyploids amongst plants and their scarcity amongst animals constitute a striking difference between evolutionary patterns in the two kingdoms (Dobzhansky 1970). The principal reasons are: (i) that the higher animals are mainly bisexual with sex chromosome mechanisms, which would be effectively destroyed by polyploidy; and (ii) in the hermaphroditic groups, cross-fertilisation is obligatory or near-obligatory, as a result of which a new polyploid will only be able to obtain a diploid mate for reproduction, producing sterile triploid progeny. Another barrier was suggested based on the greater histological complexity of animals than plants. However the occurrence of polyploidy in parthenogenetic animals refutes this suggestion.

In nematodes, *gynogenesis* is a type of pseudo-fertilisation. A sperm enters an oocyte, activates it for further development but degenerates later without fusion. During heterogony in the same group parasitic generations alternate with free living generations.

Amongst the beetles, three types of parthenogenesis are known: (1) arrhenotoky (sex determination by haplodiploidy); (2) deuterotoky (both sexes produced parthenogenetically); and (3) thelytoky (including pseudogamy). *Gonomery* is the tendency of one or more whole sets of chromosomes to become separated

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*(See Sharma AK and Sharma A 1983 ed *Chromosomes in evolution in eukaryotic groups* vols 1 and 2, CRC Press, Boca Raton, FL, USA)

from the rest at the metaphase of the egg.

A few records are available of tetraploid members in the hermaphroditic Turbellaria and in freshwater members of Basammatophora. On the other hand, parthenogenetically reproducing animals like members of the order Hymenoptera show endopolyploidy in the somatic tissues of the otherwise haploid males. Amongst the animals reproducing through thelytoky—whether *automictic* or *apomictic*—where males are genetically non-functional and individuals develop from eggs, numerous species of weevils belonging to the sub-families Otirrhynchinae and Brachyderinae are known to be triploids, tetraploids and in some cases, pentaploids. The association of parthenogenesis with polyploidy can also be seen in parthenogenetic insects, crustaceans, earthworms and vertebrates. No evidence for evolutionary polyploidy has been obtained in the dipterous flies, orthopteroid insects and mammals. In the frogs and toads, however, polyploid species and races have been observed, a well-known case being the South American toads of the family Ceratophrydidae with $2n$, $4n$ and $8n$ races. The sex determination mechanism in these forms probably depends on the dominant Y principle—with one sex in the tetraploids XXXY rather than XXYY.

Parthenogenesis has been recorded in 26 species of lizards and one each of snake and iguanid. It has resulted either following hybridisation in ecotonal habitats or through gene mutation or recombination. Polyploidy is rare and sometimes arises through hybridisation of parthenogenetic individuals. In *reptiles* (except in advanced snakes), the sex chromosomes are in early stages of differentiation. In environmental sex determination, the sex of a juvenile is determined by a particular environmental parameter, like temperature. In genotypic sex determination the sex is determined by some gene or genes inherited from either parent at the time of fertilisation. Many species, showing the latter, have morphologically differentiated sex chromosomes, a male heterogametic XX♀/XYO or a female heterogametic ZZO/ZW♀ Multiple sex chromosome systems, arising from sex chromosome-autosome translocations, are known in both lizards and snakes. Genotypic sex determination in this group has evolved independently involving different chromosomes and perhaps even different genetic systems.

The mammalian X-chromosome has been regarded to show conservatism presumably due to its involvement in dosage compensation. However, the avian Z chromosome shows an equal conservatism *in toto* though dosage compensation is regarded as absent in birds.

HOMOSEQUENTIAL SPECIES: Identical karyotypes in different species have been recorded in some cases. For example, in the *Drosophila* fauna of Hawaii, the related species appear to have completely identical karyotypes, as observed from a band-by-band comparison of the polytene chromosomes from the salivary glands. However, such *homosequential* species are rare and with improved techniques, involving DNA measurements, autoradiography and the differential banding patterns, it may become possible to detect the probable very minute chromosomal alterations acting in their evolution

MINOR CHROMOSOMAL CHANGES: Minute chromosomal rearrangements, as discussed in an earlier chapter, have played a highly significant role in speciation, both in plants and animals. Their presence is indicated by the variations in DNA values, in the amount and distribution of heterochromatin and in the quantity of repetitive DNA observed in related species.

Amongst nematodes, the haploid genome of *Caenorhabditis elegans* gives an estimate of $0.8 \times 10^8$ base pairs of DNA—apparently the smallest amount of DNA observed in any animal. In the related genus, *Panagrellus silusae*, the haploid DNA content is four times more. An even larger amount appears in *Ascaris lumbricoides.*

The haploid mammalian genome contains $3.2 \times 10^9$ base pairs of DNA; the average distance between neighbouring genes is estimated as 35,000 base pairs. The genetic distance of one crossover unit covers approximately one million b.p. of DNA. Mammals, in the absence of polyploidy, have to rely exclusively upon tandem duplication of the already existing genes as the means of generating new genes.

These parameters have indicated that chromosomal changes have taken place in the evolution of the taxa studied, which were otherwise undetectable with the conventional techniques. Reassessment of genera through the study of these criteria has shown that animal groups, previously regarded to have very uniform karyotypes, have in reality a large number of differences between them. An example is the group Acrididae of short-horned grasshoppers where the almost universal karyotype is $2n=23$♂ acrocentrics. A careful comparison has shown the minute differences in karyotype involved in their speciation (see White MJD 1973 *Animal Cytology and evolution* 3rd ed Cambridge Univ Press, London; 1978 *Modes of speciation* Freeman, San Francisco).

A parachute association of sex chromosomes Xyp is seen in several insect species, notably *Polyphaga*. It has been interpreted to be nucleolar in nature or due to telomeric association. Because the Xyp has survived more speciation processes than any other heterosomal pairing type, it may be regarded as mechanically the most competent sex bivalent (Virkki N 1983 In: *Chromosomes in evolution in eukaryotic groups* **1** ed Sharma AK and Sharma A CRC Press, USA).

MAJOR CHROMOSOMAL CHANGES: Major chromosomal changes in the evolution of species have been recorded for several groups and more data, particularly in the mammals, is being accumulated with the application of the banding pattern techniques (see earlier chapter). Paracentric inversions have been mostly responsible for polymorphism as well as speciation within certain groups of *Drosophila*, as discussed under chromosomal rearrangements. Other types of alterations are less frequent in this group—there being a record of more than 100 paracentric inversions as against three centric fusions, one pericentric inversion and some other structural changes in the Y-chromosome. Within the chironomid midges, a number of translocations involving whole arms have been operative during evolution. The primitive *Chironomus* has chromosome arms which are variously combined into metacentrics in the different species groups. These arms can be identified in all the members of the group by their banding patterns.

*C. thummi* has been shown to contain, at particular bands, 2, 4, 8 or 16 times the amount of DNA present in the corresponding band of a closely allied species *C. piger*, indicating the direct duplication of the DNA content at particular regions during evolution. Duplications of chromosome segments have been found to accompany speciation in certain Anopheline mosquitoes. Fusion of acrocentrics has been observed in a few grasshoppers of the family Acrididae—an otherwise uniform group with $2n=23$♂. A South American member, *Dichroplus silveiraguidoi* has $2n$♂ ♀ $=8$, obtained from the ancestral numbers of $2n=23$♂ through a series of fusions, seven between autosomes and one between the X-chromosome and an autosome and six pericentric inversions. Another species in the family Pyrgomorphidae has acquired $2n$♂ $=11$ from an original karyotype of $2n$♂ $=19$ acrocentrics by four autosomal fusions. Pericentric inversions have been observed in a few members of Acrididae to have transformed some acrocentrics into metacentrics without any change in number. Within the Australian grasshoppers of the subfamily Morabinae of Eumastacidae, the primitive number of $2n$♂ $=17$ has given rise to numbers ranging from $2n$♂ $=13$ to $2n$♀ $=21$, through a set of about 39 chromosomal fusions and 22 dissociations together with other changes like pericentric inversions and alterations in amount of heterochromatin. Within the morabine grasshoppers of the *viatica* group in South Australia, speciation has taken place through the spread of numerous chromosomal rearrangements. They include fusion, pericentric inversions in the X and autosomes, X-autosome fusion and duplications in the short arms of acrocentric chromosomes, in addition to others. This method, through which a number of species arise from a single one by numerous chromosomal rearrangements, spread to occupy limited areas and act as incipient isolating mechanisms in narrow zones of hybridisation, is known as *stasipatric* speciation. In certain beetles like the American genus *Chilocorus*, *diphasic* chromosomes have been observed with one arm euchromatic and the other heterochromatic. Translocations between these chromosomes have led to the fusion of the euchromatic arms into a metacentric and loss of the heterochromatic arms. In *Chilocorus stigma*, from an original form with $2n$♂ $=11$ pairs of diphasics$+X_1X_2Y$, three different fusions have given rise to individuals homozygous for all three having $2n$♂ $=3$ pairs of monophasic metacentrics$+$five pairs of diphasics$+X_1X_2Y$.

Chromosomal rearrangements have been observed in many mammals as well (see also previous chapter). For example, in different polymorphic populations of the European shrew *Sorex* *araneus* the chromosome number ranges from $2n$♂ $=33$ to 21, due to six fusions. A very closely related species *S. gemellus* has $2n$♂ $=23$. In some cases, the karyotypes of closely related species differ widely, like the Indian muntjac deer *Muntiacus muntjac* $2n$♀ $=6$, $2n$♂ $=7$ ($X_1X_2Y$) and its close relative *M. reevesi* with $2n=46$, implying a possible 20 chromosomal fusions in the evolution of muntjac. In the African mouse *Leggada,* chromosomal fusions are frequent. Members of the genus *Equus* have chromosomes ranging from $2n=34$ to 66, although some members of Camelidae are known to have very similar karyotypes.

In Aves, with the exception of Falconiformes, most species have several pairs of macrochromosomes and a large number of so-called microchromosomes. Diploid numbers range from 50 in Falconidae to over 100 in Rallidae with modal number at around 80. Sex determination is of ZZ-ZW type (female heterogamety).

*Mammals show conservation of karyotype* in spite of variations in number which may be attributed to fusions.

*One extreme* is occupied by the order *Cetacea.* The two suborders Odontocete (toothed whales), and Mysticeti (baleen whales) existed in late Oligocene 28 million years ago. But the present day representatives of both have $2n=44$ chromosomes, nearly indistinguishable even with G-banding.

*Other extreme* is formed by the family Equidae—each species has its own unique karyotype, e.g. domestic horse ($2n=64$), common zebra ($2n=44$), onager wild ass ($2n=54$). Such extensive chromosomal rearrangements have caused surprisingly little disturbance in linkage groups. For example, the closely related Indian muntjac *Muntijacus muntijak vaginalis*) has $2n=6$ in ♀ and $2n=7$ in ♂ due to X-autosome translocation and Chinese muntjac (*M. m. reevesi*) has standard cerbid $2n=46$. Interspecific hybridisation is possible. The sterile hybrids show that chromosome 1 of the former corresponds to 8 acrocentric autosomes of the latter united by one Robertsonian and 6 tandem fusions; chromosome 2 to another 8 acrocentric autosomes and chromosome 2 (and so-called $Y_2$) of the former fused with the X and the remaining six autosomes of the latter.

In general, in spite of the apparently high diversity in karyotypes of mammals, the underlying theme is the conservation of linkage relationships between genes. However the mammalian euchromatic region has very few still functioning genes scattered over a barren stretch.

KARYOTYPE ORTHOSELECTION: In the different groups of animals, there is a tendency for the repeated occurrence of the same type of chromosomal alteration during evolution. This phenomenon is referred to as *karyotype orthoselection*. As a result, within a particular group, the karyotypes show a systematic uniformity and the chromosomes of a taxon are either similar or can be grouped into two quite distinct types. An example of the latter type are the reptiles most of which exhibit two general types of chromosomes, the large macrochromosomes and the small microchromosomes. The *viridis* group of species from *Drosophila* has involved in its speciation, more than 100 paracentric inversions, as mentioned earlier. Similarly translocations between diphasic chromosomes have been the principal means of speciation in *Chilocorus*. *Lysandra atlantica* has acquired about 195 dissociations and the Algerian race of *Gerbillus pyramidum* 19 fusions. This tendency is observed in certain groups of plants as well though it is not as frequent as in the animals.

In *Ascaris* the large multicentric chromosomes are present only in germ line cells. They undergo fragmentation and give rise to a number of smaller chromosomes in the somatic cells during cleavage. They are believed to have evolved from end-to-end association of smaller chromosomes which may have a single centromere or a diffuse one. This association must have occurred several times.

The end result of the repeated occurrence of the same type of structural rearrangement during the evolution of a particular group is an order and symmetry in the karyotypes of the great majority of the animal species, which would be lacking in the absence of karyotype orthoselection. White (1973) has suggested two separate ways through which selection may have canalised chromosomal alterations in speciation. Changes of the same type within a species may have the same kind of physiological and phenotypic effects. An accumulation of these changes may tend to adapt the organism to a particular environmental selection. Taking the previously cited case of the mollusc *Thais lapillus*, the transformation of the $2n=36$ race to the $2n=26$ one, through five successive fusions may have progressively adapted the individual to the specific ecological conditions—increased waves and abundance of food. Orthoselection may be caused by internal factors as well, for example, the necessity of chromosome shape and size to remain harmonious with the spindle and cell size. An alternative, and subtler means for the operation of orthoselection, is through the distribution of chiasmata. The pattern of chiasmata localisation is similar within the chromosomes of a complement. This similarity often extends to the karyotypes of related species as well, as for example, the chromosomes of most spiders tend to show extreme proximal localisation. These resemblances in chiasmata pattern may have arisen through karyotype orthoselection and they themselves lead to further orthoselection by determining the type of rearrangement possible.

Speciation within the animal kingdom, according to White (1973), has been a gradual process. The formation of new races, whether through genic changes or chromosomal rearrangements, constitutes an early stage of species formation. Cytogenetic evidence appears to indicate that in cases where chromosomal rearrangements have been directly involved in them, their role is exerted from an early stage during the evolutionary history onward. The final steps take place through the gradual collection of polygenic changes. Their cumulative effect increases the strength of genetic isolation until it becomes complete, giving rise to a new taxon.

CHAPTER V: 3

## BIOLOGICAL SPECIES CONCEPT

### A. In sexually propagating eukaryotes

DEFINITION: Taxonomy is the science of affinities, endeavouring to propose a scheme of classification for the living organisms, which would mirror, as efficiently as possible, the phylogenetic relations uniting different groups and the similarities at each taxonomical level. The unit of taxonomical grouping is usually the *species*. The definition of this term, since its inception, has been variously modified by various workers, depending on the criteria stressed by them, even to the extent of suggesting that it may require several definitions. Biosystematics, on the other hand, concerns itself primarily with the processes of evolution and only secondarily with its end products. It involves differential analysis of the causes of differences between members of a related group and also between related groups, through a synthesis of data from different branches. Since its advent the biological species concept has gained prominence.

According to this concept, species may be defined as "groups of interbreeding natural populations that are reproductively isolated from other such groups" (Mayr E 1969 *Principles of systematic zoology* McGraw, New York). Another allied definition regards the species as "the sum total of the races that interbreed frequently or occasionally with one another, and that intergrade more or less continuously in their phenotypic characters" (Grant V 1963 *The origin of adaptations* Columbia University Press, New York).

MISCIBILITY BETWEEN POPULATIONS: The species, outlined on the basis of this concept, is viewed as a natural population or a system of populations, composed of genetically closely related individuals. Taxa other than species are composed of schetically related individuals. The boundary between species is due to genetic barriers between populations which do not mix and do not lose their identities on being grown in close proximity. Thus miscibility between populations and not crossability is the important factor in evolution. A new species arises when a major genetical system becomes closed and loses its ability to interbreed and fuse with other such systems. Two populations, which have reached the rank of species, will not mix if grown side by side under natural conditions. Cytotaxonomic studies on higher animals confirm the biological species as distinct entities which generally do not intergrade.

PROBLEMS: The problems involved in accepting this concept include: (i) the possible means through which the gaps between incipient species develop; (ii) the ways through which such gaps

**TABLE V : 3.1**
**Modes of speciation**

| *Types of speciation* | *Basic mechanism* |
|---|---|
| 1. Divergence | |
| a. Adaptive | Erection of extrinsic isolating barrier followed by independent microevolution |
| b. Clinal | Selection on a cline with isolation by distance |
| c. Habitat | Selection over multiple habitats with no isolation by distance |
| 2. Transilience | |
| a. Genetic | Founder event causing rapid shift in previously stable genetic system |
| b. Chromosomal | Inbreeding and drift causing fixation of strongly underdominant chromosomal mutation |
| c. Hybrid maintenance | Hybridisation of incompatible parental species followed by selection for maintenance of hybrid state |
| d. Hybrid recombination | Hybridisation of incompatible parental species followed by inbreeding and selection for stabilised recombinant |

**Source :** *Mechanisms of speciation—a population genetic approach,* by AR Templeton, *in* Annual review of ecology and systematics, 12 : 23-48, 1981, edited by RF Johnstone, PW Frank and CD Michener, Annual Reviews Inc, Palo Alto, Calif.

are maintained; and (iii) the genetical processes operating in the separated populations (Table V:3.1).

The primary factors involved in the development of a genetical barrier are changes at the gene and/or at the chromosomal level. Speciation in this manner may be gradual or instantaneous.

GRADUAL SPECIATION: The process of gradual speciation occurring within a population is caused by a number of changes, usually at the genic level, progressing successively through gene mutation, genetic recombination and natural selection, without usually any major change, as for example, a change in the number of chromosomes. These slow changes' include, in addition to gene mutations, the different forms of chromosomal rearrangements, which ultimately lead to loss of chromosome pairing and sterility barrier. Such gradual and additive chromosomal alterations, supported by hybridisation and natural selection, result in gradual reproductive isolation and finally in morphological discontinuities by the ordinary processes of subspeciation and extinction of intermediates. Through continuous recombination and hybridisation, a new form may arise which is genotypically stable and best suited for a particular environment. It may be regarded as an incipient species. When this form finally acquires genetic barrier, preventing its hybridisation with similar other stable forms, it may have reached the status of an independent species. Repetitive DNA may play a significant role in genetic isolation through reducing chiasma formation. According to the biological species concept, where populations are the units of evolution, they may drift apart decisively through an accumulation of such minute genetic differences. Such gradual speciation is accepted as the norm while instantaneous speciation is an exception. Gene mutations usually do not direct the course of evolution or form the immediate source of variation on which natural selection may act unless these lead specifically to barriers in chromosome pairing. On the other hand, they replenish the gene pool, substitute for the genes being lost through natural selection and these are reshuffled into the adaptive gene blocks through recombination.

INSTANTANEOUS SPECIATION: Instantaneous speciation involves the production of individuals reproductively isolated from the species to which the parents belong. An abrupt and instantaneous barrier is created to reproductive miscibility, usually by a change in the chromosomal number, either through segmental interchanges or through the loss or addition of individual chromosomes. In sexually reproducing plants, polyploidy, associated with hybridisation, forms a frequent means of altering the chromosome number, leading to speciation. A variant form is *agmatoploidy*, or change in chromosome number without any change in the total amount of chromatin matter. Well-known example of such a phenomenon is the genus *Luzula* of the family Cyperaceae, where the centromere is diffuse and the chromosomes may be broken into fragments, each acting as an independent chromosome. Autopolyploidy, by itself, is an evolutionary factor of some importance amongst the cultivated plants. Amongst the animals, however, the possibility of instantaneous speciation through chromosomal rearrangements has been strongly criticised by Mayr (1963). The prevalence of polyploids among plants and their scarcity among animals have led to a striking difference between evolutionary patterns in the two kingdoms.

The relative roles of genetical and geographical barriers in speciation are not mutually exclusive. The orthodox view is that speciation occurs by slow genetic divergence and subsequent reproductive isolation of geographically separated and differentially adapted races or subspecies (Dobzhansky Th 1970 *Genetics of the evolutionary process* Columbia, New York). It may also occur without any prior divergence within a relatively small number of generations by joint fixation of co-adapted genes in the population (Carson HL 1973 In: *Genetic structure of populations* **274**: 280 Univ of Hawaii Press).

GEOGRAPHICAL AND GENETICAL BARRIERS: Geographical barrier certainly facilitates gradual speciation, since *allopatric* species, separated for long periods of time, ultimately accumulate sufficient gene changes to form a genetic barrier if grown together later. Speciation in its simplest form occurs when a population becomes completely isolated geographically from the parent population. Genetic changes arise within the population, owing to mutation, recombination and random fixation, strongly influenced by selection pressure. As a result, reproductive isolating mechanisms are developed, the population diverges due to ecological requirements and genetic changes occur to make the hybrids of the incipient species with the parental one inferior in competition with either. Thus a new species develops if a population, which has become geographically isolated from its parental species, acquires during the period of isolation characters which promote or guarantee reproductive isolation when the external barriers are eliminated. Such a process in animals, may take half a million years or more.

Instantaneous speciation is responsible for the origin of *sympatric* species by segregation of individuals within a single population through a major chromosomal change which creates a genetical barrier. The sudden change may involve the number of chromosomes, so that reproductive isolation is not gradual but abrupt.

INCOMPLETENESS OF ISOLATION: Isolation between populations of a species is rarely complete. It depends principally on the distance and the completeness of the barrier. Gene flow between populations occurs to an extent inversely proportional to the distance and formidability of the barrier. In considering the barrier of segregation as compared to isolation, no two populations of the same species can co-exist in the same locality without intermixture. Conversely, when different populations of a species are spatially separated, they may interbreed in the zone of their contact, but their centres of distribution will be different. The specific status of an "allopatric" or spatially segregated species can be confirmed in relation to its ancestral species, only when populations of the two are grown together and do not intermix. On this criterion, specific rank attributed to taxa on geographical isolation alone should be re-assessed.

LIMITATIONS OF BIOLOGICAL SPECIES CONCEPT: The limitations faced by the definition of a biological species, as given earlier, are two-fold:

(i) The criterion of intersterility between the incipient species and its parent is ambiguous. The incipient species will always involve a residue of borderline cases, for which the decision will be arbitrary since species are not fixed but evolving. A morphological species, by this definition, will be an inference as to the most probable limits of the genetic species.

(ii) The process of speciation has been visualised in sexually reproducing organisms. But certain groups of animals reproduce parthenogenetically and amongst the plants, a high proportion is partially or wholly apomictic, reproducing through vegetative means. This concept of speciation does not apply to any significant extent to these forms, for which a special type of speciation has been visualised in asexually reproducing plants, as given in the following paragraphs.

### B. Speciation in vegetatively reproducing plants*

SPECIAL MEANS: In almost all the types of chromosomal alterations discussed, these changes, whether they occur in somatic or germinal tissue, must reach the germ cells to become effective in speciation. In some cases apomixis has propagated such changes. Bud mutations may arise but they have to be maintained through sexual reproduction. In normally sexually reproducing plants—including a majority of the angiosperms—any chromosome change, if it can reach the germ cell and if it is not drastic enough to cause non-viability of the gametes, can give rise to new species. But numerical alterations of chromosomes—unless involving polyploidy—usually result in non-viability of gametes and are lost in sexually reproducing plants. In plants reproducing through vegetative means—which include most of the monocotyledons, as well as certain dicotyledons propagated through cuttings—a different problem is faced. Since sexual reproduction is of secondary importance or often absent in them, obviously there is no question of chromosomal alterations being maintained through gametic propagation. A number of these plants, like the Aroids, are cultivated for their specialised leaves and flowering is scarce. In others, like members of Liliaceae and Amaryllidaceae, flowers are formed but meiosis may be irregular or the pollen sterile and thus no seeds are set. The only explanation seems that these genera have reached a static step in evolu-

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*See Sharma AK 1956 *Caryologia* **9**: 93; 1974 Pres add. Botany Sect. Indian Science Congress Session; Sharma AK and Sharma A 1959 *Bot Rev* **25** : 514).

tion and have lost their capacity for speciation with the loss of sexual reproduction. This cannot be correct as these genera are dynamic and successful ones, producing numerous new variants, both in nature and under cultivation. Therefore they must have an effective means of speciation unrelated to sexual propagation.

SOMATIC VARIATIONS: In 1950, an interesting phenomenon was noted in *Caladium bicolor*—a member of the family Araceae. It has a number of different varieties, all of which showed a somatic chromosome number of $2n = 28$. In the somatic tissue, in addition, cells were observed which had the number or the structure of chromosomes varying from the normal one. The number and the karyotype occurring in the highest frequency were taken as normal for the particular individual. Within cells of the same tissue, the chromosome complement is not fixed but varies in the structure or number or both of chromosomes from the other cells. These variations occurred at random without any zonation.

On studying the different varieties and allied species, each of them were seen to have a distinct karyotype, but variants from the normal occurred in a large number of somatic cells. Significantly enough, an abnormal karyotype in one variety was found to be the normal karyotype of another variety. Therefore it was suggested that if an abnormal nucleus, with a numerically or structurally altered karyotype enters into the growing apex which gives rise to a daughter shoot through vegetative means, the new shoot will be formed of cells containing the altered karyotype, thus giving rise to a completely new form. Since sexual reproduction is absent, such abnormalities are not lost through gametic inviability.

Variation in somatic complements had been earlier observed by different workers in individual plants, but its significance was not understood. Later, the investigations were extended to almost all families of flowering plants which propagate, partly or entirely, through vegetative means (see Research Bulletin I and II, Sharma AK 1973, 1974). Uptodate, more than 600 to 700 taxa belonging to almost all groups of monocotyledons, have been studied from this aspect. In all, in addition to nuclei with normal karyotype, abnormal nuclei containing structural or numerical alterations of chromosomes, or both, have been observed in the somatic tissue. Often in related species and varieties, the abnormal karyotype of one becomes the normal one of another. In *Hemerocallis manorama*, individuals of a population collected from the Eastern Himalayas had $2n=22$ chromosomes. $2n=24$ chromosomes were recorded in a few cells in certain individuals. After several years, an individual from the same clone exhibited different floral morphology. On examination, most somatic cells in it were found to possess $2n=24$ chromosomes, which have evidently arisen from the original set of $2n=22$ through chromosomal alterations. A similar case was observed in *Agave* as well.

Reports of variant chromosome numbers for the same species from different regions and at different periods possibly provide further support of this theory. For example, populations of *Hymenocallis calathina* and *H. littoralis* studied in the author's laboratory have shown $2n=74$ and 44 chromosomes respectively as against earlier reports of 60 and 46 by Sato. In *Scilla autumnalis,* the different somatic numbers reported were $2n=24$ to 28 (Heitz 1926), $2n=29$ and $2n=28$ in the author's group. The widespread occurrence of such variations in chromosome number and structure within the karyotypes of cells of a single individual, between different individuals of the same population and different populations of the same species reproducing partially or wholly through vegetative means, can explain their role in speciation. Such variations have also been recorded in plants propagated through cuttings, like *Bougainvillea* and *Codiaeum* and in other genera where sexual reproduction is present but is also partly supplemented by vegetative propagation, like *Piper* and *Mentha.*

MODE OF ORIGIN: Taking the occurrence of such variations for granted—which militates against the concept of constancy in number and structure of chromosomes in each cell of an organism—what is the mode of origin of such variations?

Variations in chromosome structure may arise through any of the chromosomal rearrangements outlined in an earlier section, including deletions, duplications, inversions and segmental interchanges. Dissociations and centric fusions have not yet been recorded but the application of banding patterns may show up many more minor rearrangements in the karyotypes at present regarded as similar.

Variations in chromosome number may arise through non-disjunction, somatic reduction and possibly partial endomitosis. Non-disjunction, in the somatic tissue, involves unequal anaphasic separation, which results in unequal distribution of chromosomes in the daughter nuclei.

Somatic reduction has been observed widely in vegetatively propagating groups. It was induced artificially in somatic tissue originally through the use of sodium nucleate and later through several other chemicals. Disturbance in the nucleic acid balance results in reductional separation of chromosomes within the somatic tissue. This phenomenon is responsible for the origin of varying numbers of chromosomes since such division may be unequal. An irregularity induced, whether through gene mutations or chromosomal rearrangements, may give rise to successive irregular cells, upsetting the nucleic acid balance and facilitating further irregular division.

In some vegetatively reproducing plants, changes in chromosome number may involve certain sets or blocks of chromosomes only. In *Zephyranthes mesochloa*, belonging to family Amaryllidaceae, the normal somatic number is $2n=48$, but variant cells within the same tissue contain $2n=24$, 42, 54, 60, 66 and 72 chromosomes, all being the multiples of the number six. Obviously the changes involve a set of 6 chromosomes, an observation which can be explained through partial endomitosis. During interphase, these 6 chromosomes undergo one or more endomitotic reduplication, involving replication of the chromosome thread without coming to metaphase. On the initiation of metaphase, the cell would contain as many extra multiples of the set of 6 as the number of endomitotic divisions. An analogous case is the observation of endoreduplicated segments by Lejeune and Berger in human chromosomes, as mentioned in an earlier section. Such segments have been observed in karyotypes of individuals with malformed offspring, having given rise to aneusomie de recombinasion.

Since polysomaty or the occurrence of somatic cells with varying karyotypes, is a frequent feature of plants reproducing through vegetative means, the mechanism leading to this phenomenon must be under genic control. Whether observed within the sector of a root, entire root or entire bulb, it may have arisen through gene-environment interaction in adaptation to environmental disbalance. As in vegetatively reproducing plants, it leads to the origin of new genotypes which can result in the origin of new species, the genes for susceptibility to change by gene-environment interaction will have a selective value in evolution. Such minute disbalance in equilibrium between cell-environment relationship, possibly at the molecular level, is likely to be a recurrent one but the comparatively resistant genes will be less affected. Structural variations include, in a large number of cases, known heterochromatic segments, as for example, secondary constriction regions in the Liliaceae. The higher susceptibility of these regions to external agents may be responsible for this phenomenon. The application of banding techniques, for the specific detection of other heterochromatic segments, may highlight their possible role in speciation in such plants. To some extent, the maintenance of such variant cells depends on the habit of the plant. They are more frequent in marsh-loving and aquatic genera with abundant food supply where there is a little competition between normal and variant forms.

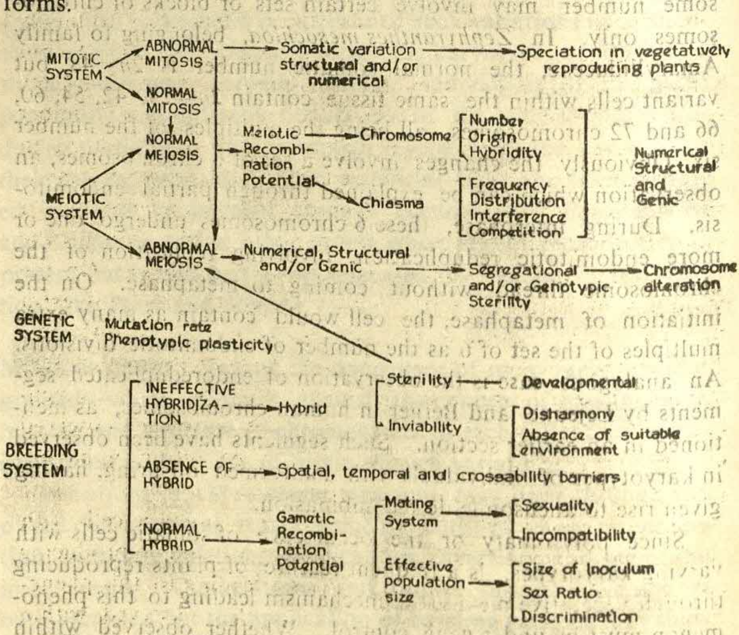

Fig. V : 3.1 The components of the genetic system (modified from Lewis and John 1963)

PROBLEMS: Various problems arise in view of this apparently irregular behaviour of plants propagating through vegetative means.

(a) The major problem is that how is the ratio between the nuclear and cytoplasmic contents of a cell—which is essential for the functioning of the cell—maintained with such alterations occurring in chromosome matter of the cell? The nucleo-cytoplasmic ratio is very subtle and its disbalance is apt to upset the metabolism of the cell. It has been suggested that possibly in vegetatively reproducing plants, the balance between the nucleus and cytoplasm is maintained in a way different from the sexually reproducing plants. Here, the balance may be maintained between the entire nuclear matter and the entire cytoplasmic content of the tissue taken as a whole instead of that within individual cells. Thus increase in chromatin matter within one cell will be offset by a decrease in another neighbouring cell and the equilibrium between the nuclear and cytoplasmic matters of the tissue as a whole will not be affected. The validity of this assumption may be tested through a quantitative estimation of the cytoplasmic RNA and nuclear DNA of the entire tissue. Such disbalance at individual cell level is advantageous to such plants, where it again contributes to further irregularities leading to somatic mutation.

(b) The next question is as to why flowers are produced in some groups of vegetatively reproducing plants but not in others? This problem has been initiated by human selection. In plants cultivated for their showy flowers, like the lilies, somatic mutations which do not possess the capacity for flowering are eliminated in horticultural selection. In Aroids, cultivated for their foliage, flowering is neglected and only mutants with showy leaves are cultivated. The species reproducing through vegetative means can be divided into three categories, those which do not produce flowers, those which produce flowers without appreciable seed set and those which propagate both through sexual and asexual means. The origin of these three categories has been attributed to artificial selection.

(c) A last problem is why varieties or species are found to differ mostly in chromosome structure but much less in number. It can be explained by the fact that structural variations have a better chance for survival than the numerical ones as they are closer to the normal condition.

Whether these aberrations, accumulating through ages in the somatic cells, have upset the balance of normality, resulting in the non-production of viable gametes and led to the adoption of the vegetative means of reproduction or whether vegetative reproduction has eliminated the necessity of germ cells and so permitted these abnormal cells to survive and to take part in speciation, are phenomena as yet unexplained.

Such chromosome mosaicism arising in response to the need for speciation proves the invalidity of the concept of absolute karyotype constancy. Rather it illustrates that dynamism in chromosome behaviour exerts a significant control on speciation (Sharma AK 1972 In : *Chromosomes today* 3 : 248).

This hypothesis, of speciation through the entrance of variant nuclei in daughter shoots in vegetatively reproducing plants, is at variance with the idea postulated (Stebbins 1971) that apomictic complexes will not give rise to new genera or families. In support of this idea, Stebbins has cited the grass genera *Poa* and *Hieracium*, "where the apomicts belonging to different subgenera have completely different mechanisms for circumventing meiosis. This suggests that in these genera apomixis has arisen more than once and that these apomicts have not been able to extend their patterns of variations beyond the confines of a single section or subgenus". In the polyploids, he has observed that apomicts are doomed to generate variants on an original theme alone, which would survive only as long as some of them are still adaptive in same habitat. This contention is definitely limited, in view of the overwhelming amount of evidences accumulated indicating the role of somatic chromosomal alterations in speciation within apomicts. In fact, the reasons for the evolution of the grasses as the most successful and widespread genera in the plant kingdom may be sought in the wide prevalence of apomixis within them. Genera within the family Gramineae, in addition to sexual reproduction, show both sexual (parthenogenetic) and asexual forms of apomixis. As a result structural recombinations and numerical alterations, including polyploidy at the germinal line, can be maintained through apomixis to overcome the sterility barrier. In addition, a change in the somatic cells can be carried on to the germinal cells and maintained, on the female line, through the direct development of parthenogenetic forms without reduction or segregation. At the same time, changes in the somatic cells can be propagated to give rise to new forms through asexual clone formation as given in the hypothesis outlined earlier. The simultaneous operation of the three modes of speciation—through recombination in sexually reproducing forms and through the maintenance of somatic variations by the adoption of sexual and asexual apomixis, has given the grasses, the flexibility and the wide range of types required for their distribution all over the world in all environmental zones.

CHAPTER V: 4

# CYTOLOGY AS AN AID IN PLANT TAXONOMY

EXPERIMENTAL TAXONOMY: In the study of evolution, phylogeny and classification of plants, the importance of cytological study has been widely appreciated. In new systematics, based on experimental taxonomy of Tischler, elaborated by Clausen, Keck and Hiesey, evidences are pooled from different avenues of research, such as ecology, morphology, anatomy, cytology, physiology, chemistry and data from fossil plants. Systematic botany no doubt provides the essential clue to ancestry, but it must be supplemented through varied evidences (see Sharma AK 1969 In: *Chromosomes today* **2**: Oliver and Boyd, London).

Classification of angiosperms in general, and monocotyledons in particular, has been a subject of considerable debate. Conflicting views have been held not only with regard to the broader subdivisions, but also in relation to the position of the minor taxa.

APPLICATION: In the two principal systems of classification, those of Hutchinson and Engler and Diels, the positions of Pandanales on the one hand and of Alismatales and Butomales of Helobieae on the other, are significant. Engler and Prantl considered Pandanales, including the three principal genera, *Pandanus*, *Typha* and *Sparganium* as the ancient stock from which the monocotyledons have been derived. Hutchinson, on the other hand, suggested that the entire monocotyledons should be traced to Alismatales and Butomales, the two orders of the primitive Helobial complex. In this system, habitat of plants has been considered to be an important phylogenetic criterion for classification, and Pandanales, from which Typhales was excluded, is regarded as related to Palmales on that basis

Therefore, the two systems differ not only in relation to the position of Pandanales, but also in the delimitation of this order.

The application of the principles of karyotype evolution in Pandanales has yielded interesting results. In both species of *Typha* and *Sparganium*, the chromosome number is $x=15$, whereas in *Pandanus*, it is a multiple of 15, that is, $x=30$. The similarity in chromosome numbers of the three genera is indeed remarkable. Moreover, in the morphology of the chromosomes, homogeneity is evident, all being characterised by very small chromosomes, with nearly identical constrictions. It is very difficult to distinguish them on the basis of their karyotypes as they look very similar to each other. Therefore, so far as evidences from chromosome studies are concerned, the inclusion of all of them under one order Pandanales, as done by Engler and Prantl, is justified.

Hutchinson, on the basis of the nature of their habitat, has separated Engler's Pandanales into two orders—Pandanales including *Pandanus* and Typhales including *Typha* and *Sparganium*, the former being nearly terrestrial and the latter being free floating aquatic types. Outstanding cytological similarity indicating genetic relationship exhibited by the three genera reveals clearly the artificiality of Hutchinson's grouping and the futility of emphasising unduly on habitat. But, the primitive status of Pandanales as assigned by Engler and Prantl can in no way be justified. Such a high number and small size of chromosomes represent undoubtedly an advanced level of evolution. Therefore, cytological evidences provide confirmation to the homogeneity of Pandanales but in no way justify its primitive status as assigned by Engler.

On the other hand, Alismatales, specially the genus *Alisma*, has the characteristic features of an ancient genus, namely, a low chromosome number starting from $x=5$ in *A. plantago-aquatica*, long chromosomes and a symmetrical karyotype with potentiality of giving rise to all the other orders of monocotyledons. But this is not true for all the families of Alismatales and Butomales. For example, members of Hydrocharitaceae, of Butomales and even *Sagittaria* and *Limnophyton* of Alismataceae, and *Limnocharis* of Butomaceae show asymmetry of chromosome arms. Therefore, *Alisma* may be regarded to represent a primitive level, but the homogeneity of the group, Alismatales and Butomales, is debatable.

There are certain genera such as *Hydrocharis* in Hydrocharitaceae which show extreme size difference in the complement. The size variation is so extreme that it finds parallel only in Agavaceae. The inclusion of Hydrocharitaceae within this so-called primitive complex should be a point of debate. But *Alisma*, in its morphological and cytological characteristics, taken in conjunction with its habitat, may in association with other primitive genera of Helobieae, be the starting point of all other monocotyledons.

Another important and debated issue is the positions of the two families, Liliaceae and Amaryllidaceae, which differ significantly in the two systems of classification. These two families have been included under Lilifloreae by Engler and Prantl—a very advanced group in this system. The distinguishing criterion is the ovary character, being superior in Liliaceae and inferior in Amaryllidaceae. Hutchinson, however, considered the inflorescence character to be more important and on the basis of the umbellate scapose inflorescence distinguishing his Amaryllidaceae, transferred the tribes Agapantheae, Gillesieae and Allieae, to this family. More important in his system is the creation of a new order Agavales, to which several genera from Liliales, such as, *Agave*, *Funkia*, *Yucca*, *Dracaena*, etc. have been transferred along with *Polyanthes* of Amaryllidales, specially on the basis of the arborescent habit, non-bulbuous rootstock and spicate inflorescence.

The creation of Agavales in Hutchinson's system of classification was undoubtedly a bold approach towards reorganising the taxonomy of these families. The treatment was entirely unorthodox in the sense that not only the established characters delimiting the two families were broken down but at the same time a new family was created with genera being taken out from the two different families.

In Liliales, primitive characteristics are present in several genera, such as *Ornithogalum* ($n=3$), *Bellevalia* ($n=4$), etc., supporting Hutchinson's stand. The creation of Agavales also finds full support from cytological observations. It is remarkable that the genera *Yucca* and *Agave*, taken from Liliaceae and *Polyanthes* from Amaryllidaceae, possess very similar karyotypes. All of them show a deep-seated chromosome number of $x=30$, and an extreme size difference within the complement, with 10 long and 50 very short chromosomes. The morphology of short chromosomes too is nearly identical in all these taxa. Most of the chromosomes possess constrictions near the tip and metacentric chromosomes are very rare. Such identical morphology makes all these taxa uniform or homogeneous in grouping. However, *Funkia*, earlier retained by Hutchinson under Liliaceae, should be transferred to this group as well on the basis of its extremely asymmetrical karyotype.

But Hutchinson also included *Dracaena*, *Cordyline* and *Sansevieria* in one tribe Dracaeneae under this family. This grouping, however, cannot be recommended on the basis of their cytology. All of them not only possess chromosome numbers ($n=18-21$), unlike any other member of Agavaceae, but the karyotype also is entirely different. They show very short chromosomes, graded in size, without the abrupt size difference which marks the other members of Agavales. Similar is true for the genus *Nolina*, the very long chromosomes of which are found to be otherwise entirely absent in all these genera. Abrupt size difference in chromosomes is the very character which groups the different genera of Agavaceae under one cytological assemblage and inclusion of any other genus not showing this characteristic makes the grouping unnatural. Thus, on this basis, Hutchinson's grouping of these three genera under Agavaceae appears unnatural.

*Sansevieria* has been placed along with *Dracaena* under Liliaceae by Engler and Prantl in the tribe Dracaenoidae. These authors further suggested an affinity between *Ophiopogon* and *Sansevieria*. Cytological similarities, as mentioned before, are so marked between the 3 genera, that their inclusion under Liliaceae, as suggested by Engler and Prantl, is corroborated. However, on the basis of external morphology, their retention under two different tribes, Ophiopogonoidae and Dracaenoidae by Engler and Prantl is rational.

All taxonomists consider the tribe Aloineae, under Liliaceae, to be a homogeneous assemblage, with three principal genera, *Gasteria*, *Haworthia* and *Aloe*. Chromosome studies agree fully with the contention of systematic botanists with regard to their homogeneity. They have the same number of chromosomes $2n$

=14. Their karyotypes are so similar as to include them even within the same genus. Marked asymmetry, with 8 very long and 6 very short chromosomes, is the characteristic feature. Here also, the nature of the constriction in both long and short chromosomes, being mostly acrocentric, is markedly alike. This is just an example where absolute cytological confirmation is provided to the stand taken up by systematic botanists.

In the family Commelinaceae, the two tribes Commelinantiae and Zebrinae showed separate trends in karyotype evolution. In the former, the karyotypes consisted of relatively short chromosomes, grading in size from principally submetacentric to acrocentric with high numbers from haploid series of $x=11$, 12, 15. In the Zebrineae, however, the numbers were multiples of $x=6$ and the species with lower numbers had a larger number of large metacentric chromosomes with progressive increase in subtelocentrics to telocentrics with increase in haploid number. In this tribe, therefore, centric fusion/fission mechanism had been involved in evolution. The divergences in karyotype of the two tribes had led to a move to separate them into two separate families. However, the discovery of a species, *Callisia fragrans*, with $2n=12$ chromosomes, six of which were metacentrics like *Zebrina* and 6 submetacentrics like *Commelina*, with graded karyotype, indicated that it might represent an intermediate type between the two tribes. Apparently the tribes have diverged in evolution from an ancestral karyotype similar to that of *Callisia*.

At the intrageneric level as well, chromosome studies have been utilised in assigning specific positions. Of the two species of *Cyanotis* growing in the Eastern India, *C. axillaris* of the family Commelinaceae showed $2n=20$ chromosomes as against $2n=24$ in allied species, including *C. cristata*. The number $n=10$ was similar to that of an allied genus *Murdannia*. However, transfer of *Cyanotis axillaris* under *Murdannia* as a species was ruled out on the basis of chromosome morphology. Therefore, *Cyanotis axillaris* was assigned a generic status, as later accepted by taxonomists (see also *Chromosomes in evolution in eukaryotic groups* vols 1 and 2, 1983, 1984, eds AK Sharma and A Sharma, CRC Press, Boca Raton, Fl, USA).

Examples of the use of cytological data in verifying or refuting taxonomic status assigned to different dicotyledonous genera include the members of Geraniales. Within this order, chromosome study does not justify the exclusion of the family Balsaminaceae, as proposed by Engler. Transfer of the genus *Averrhoa* to a different family has been justified but its inclusion under Rutales could not be supported. Similarly the creation of the family Basellaceae, including *Basella* taken out from Chenopodiaceae, by certain taxonomists, has been supported. In Vitaceae both *Leea* and *Cissus* have been regarded as primitive taxa. Similar reorientation has been suggested in Ranales, Leguminosae, Rubiaceae and Compositeae.

The cases that have been mentioned above are drawn from works carried out in the department to which the author is attached. They are only a few of a large number of taxa where cytotaxonomic studies have been carried out and limitations and advantages of the various systems and the exact evolutionary tendencies exhibited by different taxa have been outlined.

A formidable amount of data on chromosome study and its importance in cytotaxonomy can be obtained from laboratories engaged on this aspect all over the world. The information gathered is invaluable in solving problems of taxonomical dispute. However, in spite of exhaustive chromosome surveys, it is not yet possible to compile a classification of the plant kingdom on the basis of cytology alone. Such surveys are to be used in conjunction with other data, notably external morphology, to obtain a truly phylogenetic classification, based on a synthetic approach.

CHAPTER V: 15

# CHROMOSOMAL ALTERATIONS IN HUMAN EVOLUTION*

*Homo sapiens* belongs to the family Hominidae of the superfamily Hominoidea under suborder Simiae of the order Primates. The earliest known record of primates is from the palaeocene period, about 63 million years ago. Simiae evolved from the Catarrhini or old world sect during the Lower Oligocene. In the Middle and Upper Oligocene the superfamily Hominoidea emerged. It consisted of three accepted families, Hominoidae (bipedal ground dwelling man or near man), Pongidae (brachiating forest apes) and Oreopithecidae.

The karyotypes of the great apes and other primates are of special interest because of the evidence they may provide regarding chromosomal changes involved in the evolution of the human species. The living primate most closely related to *Homo sapiens* has been suggested as the chimpanzee, *Pan troglodytes*. The chromosome number of this species was recorded as 48 soon after the first correct count of $2n=46$ for human chromosomes was made. A large amount of chromosomal information was subsequently published. Comparisons were made between the human karyotype and those of other primates. These studies were, however, limited at the initial stages by the lack of definite criteria for precise identification of chromosomes, except length and centromeric index, to establish homology, either within or between species. Refinements in technique later led to the accumulation of considerable information regarding alterations in chromosome structure during hominid evolution following banding pattern analysis, the measurement of satellite DNA

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*From Datta S, Sharma A and Talukder G 1981 *The Nucleus* **24**: 1140.

and total DNA content as also amino acid sequences of specific proteins.

### Karyotypes of primates

A very large variety of chromosome numbers has been found in this group. Even in cases of numerical similarities between different groups structural alterations in chromosomes are marked. The definitely established primate chromosome numbers vary from $2n=34$ to $2n=72$ with an only common denominator of two in their distribution. This fact appears to rule out polyploidy as a mechanism of evolution (Bender MA and Chu EHY 1963 In: *Evolution and genetic biology of primates* **1**: Academic Press, New York).

The origin of the differences in the numbers of chromosomes among primates appears to be caused by alterations in the chromosomes of a basic set, with very little gain or loss of chromosomal material. Spectrophotometric measurements of the DNA content of the cells from *Macaca mulatta* ($2n=42$), *Erythrocebus patas* ($2n=54$) and *Cercopithecus aethiops* ($2n=60$) show that despite the difference in chromosome number all species have essentially the same DNA content. Principally a reduction in the basic number has been involved. In many groups of primates the karyotypes show a remarkable correlation between the total number of acrocentrics suggesting an important role of centric fusion in their evolution (Bender MA and Mettler LE 1958 *Science* **128**: 186).

#### i) *Karyotype of the superfamily Hominoidea*

All the Hominoidea studied so far show acrocentric chromosomes in different numbers (Table V: 5.1). The number of major chromosome arms in the karyotype of a species or the fundamental number (NF), regarded as more significant than chromosome number in tracing affinities, is surprisingly uniform for the three major groups of primates.

Obviously none of the existing primates can be ancestral to any of the others but some may be much closer to the ancestral forms as shown by their karyotypes. Chromosome numbers of these more primitive types tend to be higher than those of more highly specialised ones and *vice versa*. Also, the more recently

TABLE V : 5.1

Chromosome number and types in Pongidae and Hominidae

| *Species* | *2n* | *Chromosomes* | | | |
|---|---|---|---|---|---|
| | | *Autosomes* | | *Sex chromosomes* | |
| | | *Acrocentrics* | *Others* | *X* | *Y* |
| Pongidae | | | | | |
| *Pan troglodytes* (PTR) | 48 | 12 | 34 | M | M |
| *Pan paniscus* (PPA) | 48 | 12 | 34 | M | M |
| *Pongo pygmaeus* (PPY) | 48 | 18 | 28 | M | A |
| *Gorilla gorilla* (GGO) | 48 | 16 | 30 | M | M |
| Hominidae | | | | | |
| *Homo sapiens* (HSA) | 46 | 10 | 34 | M(S) | A |

evolved species usually have more acrocentrics, regardless of chromosome number, than those that evolved first. It may be due to the fact that the older forms have had more time for the inversions and translocations required to convert the acrocentrics into other types.

ii) *Karyotype of Pongidae*

A comparison of human karyotype with the karyotypes of other two primate groups indicates a greater similarity to Pongidae, which bears 48 chromosomes.

The general morphology of the chromosomes is similar in the three genera of Pongidae. The principal types are meta and acrocentrics. However, the human karyotype differs in gross morphology in the replacement of two pairs of acrocentrics of the great apes by a single metacentric pair. The number and length of acro- and metacentrics vary and do not indicate complete homology of the *Homo sapiens* karyotype with any particular genus of the apes. The Y is acrocentric in *Homo* and *Gorilla* and metacentric in *Pan* and *Pongo*.

## Comparison of human karyotype with Pongidae

i) *Banding patterns*

Analyses of differential banding patterns have enabled investigators to suggest counterparts for each chromosome of the human complement in *Gorilla*, *Pan* and *Pongo* (Table V: 5.2). Very few of the chromosomes, except the X, show exactly the same banding patterns.

**TABLE V : 5.2**

**Proposed chromosome band homologies of great apes and man (HSA) using conventional (A) and phylogenetic (B) nomenclatures (HGM4 1977)**

| *HSA* | | *PPA* | | *GGO* | | *PPY* | |
|---|---|---|---|---|---|---|---|
| *A* | *B* | *A* | *B* | *A* | *B* | *A* | *B* |
| 1 | I | 1 | I | 1 | I | 1 | I |
| 2 | II | 12, 13 | IIp, IIq | 12, 11 | IIp, IIq | 12, 11 | IIp, IIq |
| 3 | III | 2 | III | 2 | III | 2 | III |
| 4 | IV | 3 | IV | 3 | IV | 3 | IV |
| 5 | V | 4 | V | 4 | V | 4 | V |
| 6 | VI | 5 | VI | 5 | VI | 5 | VI |
| 7 | VII | 6 | VII | 6 | VII | 10 | VII |
| 8 | VIII | 7 | VIII | 7 | VIII | 6 | VIII |
| 9 | IX | 11 | IX | 13 | IX | 13 | IX |
| 10 | X | 8 | X | 8 | X | 7 | X |
| 11 | XI | 9 | XI | 9 | XI | 8 | XI |
| 12 | XII | 10 | XII | 10 | XII | 9 | XII |
| 13 | XIII | 14 | XIII | 14 | XIII | 14 | XIII |
| 14 | XIV | 15 | XIV | 18 | XIV | 15 | XIV |
| 15 | XV | 16 | XV | 15 | XV | 16 | XV |
| 16 | XVI | 18 | XVI | 17 | XVI | 18 | XVI |
| 17 | XVII | 19 | XVII | 19 | XVII | 19[1*] | XVII |
| 18 | XVIII | 17 | XVIII | 16 | XVIII | 17 | XVIII |
| 19 | XIX | 20 | XIX | 20 | XIX | 20 | XIX |
| 20 | XX | 21 | XX | 21 | XX | 21 | XX |
| 21 | XXI | 22 | XXI | 22 | XXI | 22 | XXI |
| 22 | XXII | 23 | XXII | 23 | XXII | 23 | XXII |

X, Y—same type; [1*]—This banding homology has not been completely resolved.

The human karyotype alone has a secondary constriction on the long arm. The relative length of the two arms of chromosome 1 in man is the reverse of those of *Gorilla*, *Pan* and *Pongo*. Human chromosome (HSA) 2 replaces two acrocentric chromosomes of each of the other species. Almost all the chromosomes of man and the higher primates can be derived from each other through structural alterations involving principally pericentric inversions, some paracentric inversions and a few telomeric

### TABLE V : 5.3

Relationship between human and primate chromosomes shown by *in situ* hybridisation

(Miller DA 1977 *Science* 198 : 1116)

| *Method* | *HSA* | *GGO* | *PTR* | *PPY* |
|---|---|---|---|---|
| HSA Sat I | Major site on 9, Y; minor site on 1, 5, 7, 12, 13, 14, 15, 20, 21, 22 | Major site on 2p, 9, 13, 14, 15, 16, 18, Y; minor site on 5, 22 | Major site on 2q, 7, 9, 15, Y; minor site on 13, 14, 16, 17, 18, 20, 21, 22 | Major site on 2p, 2q, 13, 22, Y; minor site on 11, 14, 15, 18, 21, ? |
| HSA Sat II | Major site on 1, 9, 16, Y; minor site on 14, 15, 17, 21, 22, X | Major site on 2p, 9, 13, 14, 15, 16, 18, Y; minor site on 22 | Absent | Major site on 2p, 2q, 13, 15, 21, Y; minor site on 10, 14, 18,? |
| HSA Sat III | Major site on 9, 15, Y; minor site on 1, 5, 13, 14, 20, 21, 22 | Major site on 2p, 9, 13, 14, 15, 16, 18, Y; minor site on 1, 5, 22 | Major site on 7, 9, 15, Y; minor site on 1, 2q, 13, 14, 16, 17, 18, 20, 21, 22 | Major site on 2p, 2q, 13, 14, 15, 18, Y, ? |
| HSA Sat IV | Major site on 9, 15, Y; minor site on 1, 5, 7, 10, 13, 14, 17, 19, 20, 21, 22 | Major site on 2p, 9, 13, 14, 15, 16, 18, Y; minor site on 22 | Major site on 2q, 7, 9, 15, 17, Y; minor site on 13, 14, 18, 21, 22 | Major site on Y; minor site on 2q, 12, 13, 15, ? |
| PTR Sat A | Multiple sites | Multiple sites | Multiple sites | Multiple sites |
| rRNA | 13, 14, 15, 21, 22 | 21, 22 | 13, 14, 18, 21, 22 | Many sites |

fusions. Translocations and other complex changes are more rare (Table V: 5.3).

Deletions and duplications of chromosome segments, involving major genes, have not been identified, but variations have been recorded in certain cases.

The easiest way to make the chromosome bands match in various primate species requires pericentric inversion. Differences between man and *Gorilla*, man and *Pan* and *Gorilla* and *Pan* have been attributed to inversions involving six to eight chromosomes. Nine to ten inversions separate these species from *Pongo*. Pericentric inversions are known in human populations but they are relatively rare. They are not regarded as a major mechanism involved in speciation in mammals due, presumably, to the reduced fertility of heterozygous carriers. However, pericentric inversions in both homozygous and heterozygous states have been recorded in the great apes that are compatible with fertility and which have become widespread within populations.

The difference in chromosome number of man with the members of Pongidae could be explained by fusion of two different acrocentric chromosomes, about the length of D chromosome, to form one large submetacentric chromosome—the human number 2. Fusion of acrocentric chromosomes is well known in the present day human populations (see Vogel F and Motulsky G 1979 *Human Genetics* Springer, Berlin). The HSA 2 is apparently the result of such a telomeric fusion where the activity of one centromere has been suppressed.

Different groups of researchers, working on primate karyotypes, reached somewhat different conclusions about the evolutionary changes involved in the chromosomes. Since many of the numbering systems were based on that used for man, all of them were later combined into a standard system (Table V: 5.4). Considering each of the bigher primates as a separate species, each numbering system was derived independently on the basis of chromosome length and arm ratio. The same system was used for *Pan troglodytes* and *P. paniscus* due to the close similarity of their karyotypes. The classifications included illustrations of chromosomes stained to show G, Q and R bands and a diagram comparing the banding patterns of chromosomes regarded as homologous.

This joint report (1975) failed to homologise the Y chromosomes of *Gorilla*, *Pan* and *Pongo*, which show greater interspecific variability than any other chromosome. The banding pattern of *Gorilla* Y chromosome is more complex than that of man, which in turn is more elaborate than the two other great apes. *Gorilla* Y chromosome takes up moderately bright stain with quinacrine and differs from the human Y in having a very dull band on each arm. It is the only mammal, other than man, known to have a brilliant Q-stained region on the Y chromosome. *Pan* has a very small Y chromosome, not clearly banded by any of the available methods. Y chromosome of *Pongo* is longer than that of *Pan* but has no brilliant Q-fluorescence. The homology of genetic information among Y chromosomes can also be inferred from the spermatozoa. Through scanning electron microscopy, a close similarity in the morphology of human and *Pan* spermatozoa has been unequivocally demonstrated, while those of the other primates are considerably different.

**TABLE V : 5.4**

**Gene maps of man and great apes**

| *Species* | *Haploid number* | *Known linkage and syntenic groups* | *No. of mapped genes* |
|---|---|---|---|
| *Homo* | 23 | 23 (23) | 345 (215) |
| *Pan* | 24 | 18 (17) | 36 (31) |
| *Gorilla* | 24 | 21 (19) | 38 (32) |
| *Pongo* | 24 | 14 (13) | 26 (22) |

Report of HGM 5 (1979); The number of mapped loci does not include loci for sat DNA. Numbers in parentheses give data available at HGM 4 (1977)

ii) *Distribution of F-bodies*

The most brilliantly fluorescent region in the human karyotype is a Y chromosome, which is usually seen as F-body in somewhat less than 50% of spermatozoa.

*Pan* and *Gorilla* show brilliant fluorescent regions in some of their chromosomes. In *Pan*, they are restricted to autosomes 14, 15, 17, 22 and 23. Y chromosome is a small submetacentric, staining palely. *Gorilla*, on the other hand, shows brilliant fluorescence, both in autosomes 2, 12, 13, 14, 15, 16, 22 and 23 and at the terminal segment of the Y chromosome (Paris Conference 1971, Supplement 1975). In *Pongo*, none of the chromosomes fluoresces brilliantly.

The presence of F-bodies in the spermatozoa of *Pan* and *Gorilla* and their absence in *Pongo* can be explained on the basis of Q-banding patterns. In *Pan*, since Y has no brilliant fluorescence, the F-bodies are presumably due to autosomal material. The situation in *Gorilla* is complicated by the presence of the fluorescent regions on both Y and some autosomes. Spermatozoa have been seen with two F-bodies in *Gorilla* in 18.6 per cent cases, compared with 1.25 per cent in man. Non-disjunction of the Y chromosome is unlikely to account for this high incidence, nor could it explain the spermatozoa with three or four F-bodies using the same type of frequency distribution analysis as applied to *Pan*. The fact that 40 per cent of spermatozoa show no visible F-body, rules out the possibility of a single homozygous fluorescent region in *Gorilla*.

### iii) *Chromosomal abnormalities*

Chromosomal abnormalities, their types and frequency are often utilised in tracing the phylogenetic relationship of man and primates. In hominoid species they include structural rearrangements and segmental or complete aneuploidy. Different types of sex chromosomal abnormalities, autosomal trisomies and pericentric inversions have been reported in higher primates as well. However, records are necessarily incomplete due to biased sampling and early loss of abnormal individuals in the wild condition. A rearranged chromosome corresponding to the human 12 is present in *Pongo* with some differences in geographically isolated populations. Inversions tend to provide selective advantage to the individuals through reduction in the frequency of crossing-over, thus isolating specific groups of alleles.

Position effect may be a relevant factor in the evolution of human karyotype. It is interesting that the incidence of individuals who have a chromosome rearrangement with no apparent loss of chromosomal material is five times greater in mentally retarded populations.

iv) *Present atlas of human chromosomes*

Each pair of the human karyotype has certain specific characteristics. Certain chromosomes have remained extraordinarily stable and may be referred to as *paleochromosomes.* Chromosome 6 is a good example. On the other hand, some have undergone numerous rearrangements and in their current forms differ widely in the four genera (Table V: 5.5, De Grouchy J and Turleau C 1977 In: *Clinical Atlas of human chromosomes* Wiley, New York; Dutrillaux B 1979 *Hum Genet* **48**: 251). Complete gene mapping of the stable chromosomes might indicate the stabilisation time of particular genes during evolution.

TABLE V : 5.5

**Comparison of structural differences between the human karyotype and the karyotype of the great apes. Numbers in parentheses indicate likely but not confirmed events (Dutrillaux B 1975 Sur la nature et l'origine des chromosomes humaines. L'expansion Scientific, Paris)**

| *Comparison* | *Pericentric inversion* | *Paracentric inversion* | *Telomeric fusions* | *Other rearrangements* |
|---|---|---|---|---|
| *Homo sapiens-Pan troglodytes* | 6 | 0 | 1 | |
| *Homo sapiens-Pan paniscus* | 6 | (1) | 1 | |
| *Homo sapiens-Gorilla gorilla* | 8 | 2 | 1 | 1 translocation |
| *Homo sapiens-Pongo pygmaeus* | 7 | 3 | 1 | 1 complex change |
| *Pan paniscus-Pan troglodytes* | 0 | (1) | 0 | |
| *Pan paniscus-Gorilla gorilla* | 6 | 2 (+1) | 0 | 1 translocation |
| *Pan paniscus-Pongo pygmaeus* | 9 | 3 (+1) | 0 | 1 complex change |
| *Pan troglodytes-Gorilla gorilla* | 6 | 2 | 0 | 1 translocation |
| *Pan troglodytes-Pongo pygmaeus* | 9 | 3 | 0 | 1 complex change |
| *Gorilla gorilla-Pongo pygmaeus* | 10 | 3 | 0 | 1 translocation<br>1 complex change |

**Evidences from repetitive and sat DNA**

The recognition of different types of repeated DNA has led to the use of this parameter in studying the evolution of man and the hominoid apes. The homology between repeated DNA of various species is suggested as an exponential function of the time since their divergence, indicating that repeated DNA homology reflects evolutionary relationships. Comparative $C_0t$ analyses of DNA from a range of primates show very striking differences in the complexity of their repeated DNA. The percentage of the total repeated DNA varies from 29 in *Macaca* to 47 in *Hylobates*. However, primate genomes are almost uniform, around $3.4\times10^{12}$ daltons. To account for variation in primates such genomes may be regarded to maintain a steady state relationship. Apparently the rate of addition of new DNA to the genome has remained fairly constant, around 0.12 per cent of the human genome for each million years. A mechanism for creating such new DNA is by saltation of a short nucleotide sequence family (sat) DNA (see Gosden J *et al* 1977 *Chromosoma* **63**: 253; Gummerson KS 1972 *The evolution of repeated DNA in primates* PhD thesis, Johns Hopkins U, Baltimore).

Secondary constrictions in human chromosomes may occur on the short arms of all five pairs of acrocentrics and may come closer by association of nucleoli. 18S and 28S rRNA genes are localised in these regions. Ag-As staining shows a variable number of secondary constrictions on acrocentric HSA chromosomes. PTR and *Hylobatus* correspond exactly to those located by *in situ* hybridisation with rRNA. PPY has more and GGO has less sites stained by silver.

18 and 28S ribosomal RNA genes form a rather special class due to their presence in hundreds of copies per cell, as is true of most genes. Extensive re-distribution of the rRNA genes has occurred on acrocentric chromosomes in *Pan*, in man on five pairs and in *Gorilla* on two pairs.

Detailed comparisons show the presence of heteromorphic chromosomal regions, many of which contain highly repetitive DNA sequences. Quinacrine brilliant regions are found only in *Pan*, *Gorilla* and man, a Q-brilliant Y being present only in the latter two species.

Human DNA contains several satellite DNA fractions, of

**TABLE V : 5.6**

**Relative stability of human chromosomes during evolution**

| *Chromosome number (HSA)* | *Stable/unstable* | *Remarks* |
|---|---|---|
| 1 | Relatively stable | Similar in PPA/PTR, GGO, PPO; secondary juxtracentromeric constriction only in HSA |
| 2 | Unstable | Important in human evolution; arising through fusion of two acrocentrics of other primates |
| 3 | Unstable | Complex rearrangements; same in HSA, PTR and GGO but differs from PPY in a large pericentric or a translocation pericentric inversion |
| 4 | Unstable | Identical in GGO, PPY and PTR and differs in man in a pericentric inversion |
| 5 | Unstable | Single pericentric inversion |
| 6 | Stable | Paleochromosome |
| 7 | Unstable | Complex rearrangements |
| 8 | Relatively stable | Differs in GGO by a pericentric inversion |
| 9 | Unstable | Complex rearrangements |
| 10 | Unstable | Identical in HSA and PTR; differs in GGO by pericentric inversion and in PPY by paracentric inversion |
| 11 | Relatively stable | Identical in HSA, PTR and GGO; differs in PPY by a translocation |
| 12 | Unstable | Identical in HSA and PPY; differs in GGO and PTR by pericentric inversion |
| 13 | Stable | — |
| 14 | Relatively stable | Single pericentric inversion in GGO |
| 15 | Relatively stable | Differs only in PTR, by very small pericentric inversion |
| 16 | Unstable | Identical in HSA and PPY; differs in PTR and GGO by pericentric inversion |
| 17 | Relatively stable | — |
| 18 | Relatively stable | Identical in PTR, GGO and PPY; differs in man by a pericentric inversion |
| 19 | Unstable | Varies among genera |
| 20 | Unstable | Undergone rearrangements, differs in PPY from the others in the presence of a pericentric inversion |
| 21 | Stable | — |
| 22 | Stable | — |
| X | Relatively stable | Shows similarities and dissimilarities |
| Y | Unstable | Acrocentric in HSA and PPY; metacentric in PTR and GGO |

which three have been studied with respect to higher primate evolution. They are concentrated within centromeric heterochromatin in different proportions in different chromosomes. In the human Y, sat DNA occurs in a distal heterochromatic segment in addition to the centromere. The chromosome regions containing sat-DNA vary in size according to the amount of the latter and are inherited. The age of satellite III has been estimated to about 30 million years, of sat I 18 million and of sat II 6 million.

Human sat DNAs I, III and IV generally anneal to corresponding sequences on fixed metaphase chromosomes of great apes, although to only a small extent in *Pongo*. Human sat III is related by hybridisation to DNA sequences in constitutive heterochromatin in all higher apes except *Hylobates* showing that they share a common ancestor. The chromosomal regions involved also exhibit differential staining by the Giemsa pH 11 procedure.

Human sat-I and II cannot be identified in *Pan* by molecular hybridisation and are therefore assumed to have originated subsequent to divergence of *Pan*. This assumption is supported by the lesser divergences of sat DNAs I and II and by the fact that the chromosomal features concerned are absent from the homologous chromosomes of *Pan*. Thus chromosome I in *Pan* lacks a prominent heterochromatic block near the centromere, which contains sat II in man. If human sat II-like sequences are present in *Pan*, their amount must be very small. This finding is supported by the relatively rare occurrence of 5-methylcytosine which is abundant in human and *Gorilla* sat II. Similarly *Pan* Y chromosome does not have the distal fluorescent portion which contains a high concentration of sat I in man.

Y chromosome in *Gorilla* does show a distal fluorescent region. However, information about sat DNA in this species is relatively meagre except for the presence of a sequence related to human sat III DNA and no record of sat I is yet available.

Apparently, the known sat DNAs of man can be dated in evolution to a period not earlier than the man-gibbon divergence. Palaeontological evidence indicates it to have occurred about 30-40 million years ago. The four known sat DNAs of man contribute approximately 0.14, 0.6, 0.8 and 2 per cent of the human genome, giving a total of 3.54 per cent. This amounts to an additional 0.09-0.12 per cent of DNA per million years. This value is very close to that given for the rate of addition of new DNA to primate genomes. Probably a significantly large proportion of the new DNA, if not all, is added as simple sequence DNA. Constitutive heterochromatin obviously generates novel DNA. The fact that each species has a different and characteristic sat DNA pattern indicates a rapid spread of new DNA through a species. The simplest mechanism would be one in which new DNA was only produced or tolerated during speciation so as to be included in all of the initial members of a new species (Jones KW 1976 In: *Chromosomes Today* **5**: 305 and 309, Wiley, New York).

At other times strong selection pressure may operate against saltatory events, originating from the involvement of sat DNA with constitutive heterochromatin implicated in mechanisms of developmental homeostasis. The phenotypic changes accompanying speciation might favour alterations in constitutive heterochromatin achieving a new homeostatic balance. Thus those underlying saltatory processes may be encouraged which would lead to the generation of new sat DNA and perhaps the elimination of certain pre-existing ones. Further alterations in heterochromatin after stabilisation of a new phenotype would be disadvantageous. According to such a mechanism, evolutionary alterations in sat DNA may denote important speciation events. The occurrence of two sat DNAs with different degrees of sequence divergence and thus of different ages exclusively in man (sat I and II), may indicate at least two important evolutionary steps that have occurred during human evolution, after diverging from *Pan*. Palaeontological records support this possibility. Further quantitative variations, inherited as chromosomal polymorphism and involving quantitative differences in sat DNA on homologous chromosomes, may have led to the wide range of normal phenotypes in modern man.

Later techniques involving differential restriction enzyme patterns have made it possible to detect minute quantities of sat DNA in human chromosomes not identifiable through hybridisation. Sat sequences on different chromosomes have been suggested to have evolved independently from a common precursor. Hae III restriction pattern, characteristic of human sat III, has been observed in all members of Hominoidea but only in *Macaca* of lower primates. Hybrids of sat III with hominoid DNA are stable, indicating that this sequence has been conserved for 25-30 million years (Mitchell AR 1979 In: *Comm Rep on Human Genetic Mapping* 5).

### Evolutionary implications

Every human chromosome has been observed to have a recognisable counterpart in the four present day species of great apes. A certain amount of reorganisation and alteration of the genetic material has obviously occurred during the evolution of the higher primates and man. A fusion resulted in the human $2n=46$ chromosomes as compared to $2n=48$ in the great apes. The most frequently observed differences have arisen through pericentric inversions. However, in view of the relatively meagre information available on gene mapping of chromosomes of higher primates, this statement cannot be taken for granted. Miller DA (1980) in reviewing the work of Seuanez HN (1979 *The phylogeny of human chromosomes* Springer, Berlin) has suggested that the pericentric inversions which have become fixed in primate evolution have not affected the activity of the genes on these chromosomes.

An analysis of the chromosomes through differential banding patterns confirms the relatively great evolutionary distance between the gibbon (*Hylobates*) and the higher primates. *Hylobates* does not have any of the acrocentric chromosomes present in the great apes and man. In the evolution of the latter species, a series of pericentric inversions can be visualised to convert metacentric chromosomes into acrocentrics, as for example, for the conversion of *Pongo* karyotype into *Gorilla*. The karyotype of *Hylobates* cannot be converted through any such alterations to the other large apes, showing that it had separated at a much earlier age from the other genera.

The incidence of more than one inversions at long intervals has been postulated to account for the appearance of a polymorphic pair of chromosomes in present day *Pongo* populations. Alternatively, the marked differences between the karyotypes of *Hylobates* and *Pongo* may indicate that the chromosomes of the ancestral *Pongo* form did not evolve from those of *Hylobates* ancestor by inversion, but rather by a different mechanism, such as reciprocal translocation.

Speciation of some primates has been observed to be accompanied by reciprocal translocations. For example, chromosomes of *Macaca* and the African green monkey (*Cereopithecus aethiops*) differ by a series of centric fusions and reciprocal translocations. In addition to the differences in the general banding patterns, *Hylobates* differs from the higher primates in having a single site for 18 and 28S rRNA genes, compared to the multiple sites in the others. Such a redistribution of rRNA sites could be attributed to a series of reciprocal translocations. Another indication of separation of *Hylobates* from the great apes is that the former has few, if any, of the DNA sequences the others share with human sat III DNA. Densitometric patterns of the banded chromosomes of *Homo*, *Pan*, and *Macaca*, suggest that both reciprocal translocation and pericentric inversion have been effective in generating reproductive isolation in these genera. Chromosome studies also show that human, *Gorilla*, *Pan*, and *Pongo* chromosomes are remarkably similar. About 98 to 99 per cent of the 500 or so bands as yet identified with the banding methods are estimated to be homologous in these four species. However, *Pongo* has fewer chromosomes with banding patterns similar to those of the human than *Gorilla* and *Pan*, and no Q-brilliant regions and no terminal Q or C bands. *Pongo* has a somewhat different distribution of T-bands and of DNA sequences corresponding to the human satellite IV DNA, as well as eight rRNA sites compared to a maximum of five in the other species.

Sat DNA of *Pan* shares so many of the physical properties of human sat III DNA that the original sat sequence was presumably present in a common progenitor of both these primates. Following *in situ* hybridisation, sat III DNA is found to be not only homologous with *Pan* chromosome but a physically similar sat DNA is also present in *Macaca*. The evolutionary divergence of Man and *Pan* has been suggested to take place after the origin of the original sat DNA indicating thereby that they had a common hypothetical ancestor and were subjected to equal selective pressures in the same direction to some extent.

*Pan* was regarded as more closely related to man than *Gorilla* due to the presence of 12 chromosomes considered to have virtually identical general banding patterns. Evidence from the more restricted regional banding methods, however, suggests that *Gorilla* is more closely related to man than *Pan*. Only these two species have brilliantly fluorescing regions on chromosome 4 and the Y, a large C band on numbers 9 and 16, and regions with high concentrations of 5-methylcytosine. *Gorilla*, but not *Pan*, has DNA sequences in common with human sat II DNA. The proteins of *Pan* and *Homo* are very similar, on the other hand.

The proposed order in which the progenitors of the various primate species diverged has been suggested as *Hylobates*, *Pongo*, *Pan*, *Gorilla*, *Homo*. The time interval between the divergence of *Hylobates* and *Pongo* is greater than that between the other species. Various evolutionary schemes have been proposed, based on chromosome analysis.

A hypothetical R-banded karyotype of a primate progenitor, based on the presumptive inversions that separate the primate species, suggests that the progenitor of the human race separated before the ancestral *Pan* and *Gorilla* diverged from one another. On comparisons based particularly on the evolution of 2p and 2q in the various primates, including the pygmy chimpanzee (*Pan paniscus*), a scheme was constructed in which *Gorilla* is thought to have diverged before *Pan*. A phylogenetic tree, based on the extent of reassociation and thermal stability of non-repeated sequences of DNA from the various species shows on the other hand that *Gorilla*, *Pan* and man diverged at approximately the same time.

Paleoecological, palaeogeographical and biochemical studies in human and non-human primates have shown that the branching of apes and *Homo* from the hominine ancestral stock took place about 12-15 million years ago. Rearrangements, both at the chromosomal and the DNA levels, have been important factors in the origin of the species and their polymorphism along with associated phenomena like recombination, selection and isolation. Question of whether Darwinian selection or selective neutrality is the major force in molecular evolution could be resolved by an understanding of the basis for the reduced evolutionary rate of the primates (see Szalay SF and Delson E 1980 In: *Evolutionary history of the primates* Academic Press, New York).

The demonstration that there are chromosomes with similar banding patterns in human and great apes is of interest because these chromosomes often carry the same genes. Somatic cell hybridisation methods have been used to show that one or more genes on almost every human chromosome has a counterpart on the purported ape-homologue. In only one or two cases was a particular gene not carried by the chromosomes that had similar banding patterns in the various species. Comparison with rhesus and African green monkey chromosomes shows that genes carried by human chromosome 1 have been associated with a chromosome segment having the same banding pattern for perhaps 35 million years or more.

Observations on terminal Q and T bands, heterochromatic regions and juxtacentromeric Q bands show that the karyotype differences between the five closely related species involve principally rearrangements of genetic material. An additional mechanism seems to be *de novo* synthesis as well as loss of chromosome material. Studies at the DNA level have helped to understand the nature of such newly synthesised material somewhat better.

A tentative phylogenetic tree of the primates from the prosimians to man established from banding pattern analysis of karyotypes of more than 60 species of primates shows that the entire euchromatic material, i.e., the non-variable R and Q bands, appears to be identical in all species of monkeys, apes and in humans. Quantitative and qualitative variations all involve heterochromatin. The types of chromosome rearrangements reconstructed from species differences in chromosome structure vary from one subgroup to the next. For instance, Robertsonian translocations (centric fusion) prevail among the Lemuridae; chromosome *fissions* only among the Cercopithecinae and pericentric inversions in the evolution of Pongidae, including *Homo sapiens*.

## Comments

The data gathered so far agree in drawing a common ancestral phylogeny for man with the higher primates, due to the remarkable similarity between their karyotypes exhibited through banding pattern analysis. *Hylobates* diverged early during evolution, as shown by the absence of any acrocentrics in this genus. A series of chromosomal rearrangements, involving principally pericentric inversions, characterises further evolution of the group, leading to the formation of acrocentrics. *Homo* differs from *Pongo*, *Gorilla* and *Pan* in a telomeric fusion, reducing its chromosome number to $2n=46$, in addition to pericentric and paracentric inversions and translocation. The two principal questions posed have been: (i) During evolution, following the separation of *Pongo*, what is the sequence for the divergence of *Gorilla* and *Pan* from *Homo*? (ii) What is the role of chromosomal rearrangements in the evolution of modern *Homo sapiens* from the protohominid?

*Pan* has been regarded as more closely related to *Homo* due to the close similarity in the general banding patterns of their karyotypes, in morphology of spermatozoa and in the sat-III sequences. *Gorilla*, on the other hand, resembles man in detailed quinacrine patterns on the Y chromosome and chromosomes 9 and 16 and also in the presence of DNA sequences common with human sat-II DNA and regions with high concentrations of 5-methylcytosine.

The suggestion that *Pan*, *Gorilla* and *Homo* may have diverged from the common ancestral populations at about the same time appears to be most valid. The ancestral stock may have been polymorphic for the different chromosomal alterations separating the three genera over a long period of time. With climatic changes, gradually *Pan* and *Gorilla* were restricted in localised habitats within the rain forests. *Homo* or the protohominid, having developed bipedalism, ability to handle tools and to communicate, with the associated physical and physiological changes, was distributed over much larger areas and heterogeneous environmental conditions and evolved into the present-day man.

A comparison of the karyotypes of the modern-day representatives of these genera indicates that no major chromosomal rearrangement has occurred during the evolution of protohominid after its divergence from the other genera. Variations mainly involve sat DNA and heterochromatic fractions, suggesting their significant role in the evolution of specific human characters. The total sat DNA is 3.54 per cent, 0.09 to 0.12 being added per million years. Genes important for the phase of evolution of the protohominid into *Homo* are not known, particularly those responsible for brain development. Most human DNA does not code for the specific proteins. Evolution in the hominids may have occurred in such DNA and involved the stretches of DNA which are located between structural genes. These regions are not transcribed and have a regulatory function. Of the 50,000 odd pairs of genes supposed to be present in the human karyotype, 15 per cent are known to be polymorphic while repeated DNA constitutes 40 per cent of the human genome. This innate heterogeneity has permitted the survival of man in heterogeneous environments before the advent of the cultural evolution.

CHAPTER V : 6

# ACCESSORY CHROMOSOMES

**DEFINITION AND NATURE:** Accessory chromosome, supernumerary chromosome or B chromosome, in its simplest form, can be defined as an extra chromosome that is not necessary for the individual and not homologous to any of the normal chromosomes (Müntzing 1967). These chromosomes differ from the normal ones in their variable number, smaller size and greater degree of heterochromatisation. They may vary in number within different tissues of the same individual, between different individuals of the same population and even between populations of the same species from different regions. They are usually acro- or telocentric in nature. Submetacentrics and metacentrics have also been recorded, the latter being often isochromosomes. They do not usually pair with normal chromosomes during meiosis, though they may pair with each other without the formation of chiasmata, when present in even numbers. In some exceptional cases, like *Clarkia*, pairing with basic chromosomes has been recorded. B chromosomes of different origin are observed to have basically the same organisation, though differing in structural details.

**HISTORY:** The term was used earlier to denote a sex chromosome. Such chromosomes were first recorded by AE Longley and LF Randolph in maize in 1927-1928, who referred to them as ***B chromosomes*** as against the normal number of $2n=20$ *A chromosomes* in a normal maize plant. These chromosomes were much smaller than the normal ones and occurred in varying frequencies in different strains of maize, from 0 to 20. They were observed to be heterochromatic in nature with sticky surface and differential staining cycle. When present in larger numbers, the plant bearing them appeared to be weaker and less fertile. They were studied in detail in the rye plant at the Institute of Genetics in Lund. Two B chromosomes, known as the *standard B*, were observed in two Swedish rye varieties with $2n=14$ normal chromosomes. Primitive strains of rye collected from Central Asia had them in varying proportions in different populations. Each chromosome was observed to double its number through non-disjunction in each generation, both the chromatids, during first division of the pollen grain and sometimes in its corresponding division on the female side, being included in one pole. In the rye plants with standard B chromosomes the numerical increase is observed up to the formation of a maximum of nine chromosomes. Higher numbers than two have a deleterious effect on vigour and fertility. In mosses, these are referred to as M chromosomes.

OCCURRENCE: More than 100 instances of supernumerary chromosomes have been recorded in various orders of insects alone in addition to many animals and over 380 species of flowering plants, distributed over a wide range of families. In animals these have been located principally in the insects and snails.

B chromosomes observed in the ant *Leptothorax spinosior* range from 1 to 12 and are found almost entirely in the male germ cells. They are rare in female germ cells, being maintained in oocytes but are almost totally eliminated from nurse cells. Variation in their occurrence results from their systematic elimination from certain organs and more rarely their increased rate of replication in particular tissues. They have been recorded in reproductive cells in some species of *Poa*, *Sorghum*, *Xanthisma texanum* and *Haplopappus gracilis*, in addition to other plants. Amongst these, in *Poa alpina* they are absent in the leaves while in *Xanthisma texanum* they are present in various tissues of the shoot. On the other hand, certain plants like species of *Agrostis*, *Dactylis*, *Festuca*, *Secale* and other grasses, exhibit a remarkably constant number of B chromosomes in all tissues.

EXPLANATIONS FOR EXISTENCE: The two explanations for the continued inheritance of accessory chromosomes in natural population are not mutually exclusive. They have been regarded to exert a benign effect when present in low numbers but a harmful one in higher numbers. Alternatively, in spite of being without any benign effect, they are suggested to survive in nature through an accumulation mechanism usually in the germ line. For example, in the male mealy bug *Pseudococcus obscurus*, where two functional and two non-functional spermatids are produced from each primary spermatocyte during meiosis, the B chromosomes have a 0.88 probability of passing into the functional nucleus. In females this probability is increased to 0.5. There is an apparent selection against individuals with such chromosomes, as otherwise, on the probability ratio, they would have continued to increase in the population. This selection may operate in the male line through a reduction in the relative fitness with increase in the number of supernumeraries. Though found to be deleterious, yet the occurrence of such chromosomes through generations indicates that they must have some positive adaptive role as well. In the Australian grasshopper *Phaulacridium vittatum*, a supernumerary chromosome, partially homologous to the X chromosome, has been recorded. It is transmitted to 70% of the male offspring but to only 30% of the female from the male parent. Such "accumulation mechanisms" are mainly effective through meiosis as observed in numerous insects, including *Locusta migratoria* and *Camunla pellucida* in addition to others. An exception is the snail, *Helix pomatia*, where the supernumerary chromosomes undergo non-disjunction in the spermatogonia and the rate of division of the cells containing them is higher than the normal ones so that at meiosis the cells lacking such chromosomes are statistically deficient.

In plants, the number of B chromosomes present may increase or decrease during the development of the germ line, particularly the archesporium and/or during gametophyte development. Selective elimination of high numbers keeps their number within control. In *Crepis* and *Achillea*, the number is increased during the development of germ line. In *Trillium*, *Lilium*, *Tradescantia* and *Plantago*, univalent B chromosomes segregate preferentially to the functional megaspore in female meiosis. More frequently however, accumulation takes place at mitosis in the male gametophyte or developing pollen grain, through preferential distribution. The division involved may be the first one, when both daughter chromosomes pass into the generative nucleus, ultimately leading to sperm nuclei with two B chromosomes, instead of the original one. Such behaviour has been observed in a large number of genera, like *Festuca*, *Holcus*, *Briza*, *Alopecurus*, *Collinsia* and others. In maize, on the other hand, preferential distribution takes place in the second gametophytic division, leading to sperms with different numbers of accessories. The one with the highest number possesses a greater chance of fertilising the egg nucleus. *Sorghum purpureo sericeum* shows another unusual system. The vegetative nucleus, after a normal first pollen grain mitosis, divides further, in which accessories are distributed preferentially to the resulting generative nuclei. The accumulation of accessories through these methods in nature is obviously controlled by natural selection, which eliminates the individuals with higher numbers of such chromosomes through their relative weakness and ineffectivity in competition with normal plants.

In certain cases, increase in B chromosomes was observed in plants growing under different environmental conditions e.g. clay soil (*Festuca* sp.) and dry climates.

BEHAVIOUR AND INTERACTION WITH OTHER CHROMOSOMES: B chromosomes are usually heterochromatic, showing differential staining behaviour. The centromere may be terminal (*Tradescantia virginiana*), subterminal (*Allium stracheyii*) or median (*Secale cereale*). More than one type of centromeres may be observed within the same species as well (*Scilla scilloides*). The centromere, however, shows irregular behaviour resulting in irregular anaphasic separation and uneven segregation. Polymorphism in the external morphology and behaviour of B chromosomes has also been recorded in some species.

The aberrant anaphasic separation or non-disjunction, which is responsible for the numerical variation of B chromosomes, may lead to variations within (i) different populations of the same species (rye), (ii) different individuals in the same population (*Urginea indica*), (iii) different organs of the same plant (*Poa alpina*), (iv) different cells of the same tissue (*Allium stracheyii*). On the other hand, in the genus *Festuca*, the number of B chromosomes is always constant.

Meiotic pairing of B chromosomes shows a wide range of variations in plants. It may be homologous between two similar

B chromosomes, *without* chiasma formation, or non-homologous in exceptional cases with A chromosomes (*Clarkia*). In the latter cases the B chromosome shows euchromatic behaviour that may indicate its origin from the corresponding A chromosome. A typical pairing may involve an end-to-end association between A and B chromosomes (*Haplopappus spinulosus*). Pairing may also be for only short periods, e.g. only at pachytene (*Secale cereale*).

The degree of heterochromatinisation may range from the totally heterochromatic B chromosomes of *Anthoxanthum* sp. to the totally euchromatic ones of *Tradescantia* sp. The heterochromatic B chromosomes may exhibit allocycly. In maize, the number of *heteropycnotic knobs* on normal nucleolar chromosomes is seen to be *inversely* proportional to the number of B chromosomes. It has been attributed to a possible repression of the replication in the knobs in the presence of B chromosomes. The presence of B in germinal line and its absence in the somatic cells (*Poa alpina*) indicate a possible interaction between A and B. Extra mitotic division undergone by B chromosome in pollen grains of *Sorghum* sp. leads to pollen lethality. This phenomenon may be useful in large populations existing in limited resources. Low dosages of B chromosomes may exert a favourable effect but large dosages are always harmful.

In grasshopper, the presence of B is *directly* proportional to chiasma frequency, thus increasing the rate of recombination. In *Lolium* sp., on the other hand, presence of B is *inversely* proportional to chiasma frequency in diploid but *directly* proportional in tetraploids, indicating the effect of *dosage compensation* in eliminating the adverse effect of B.

CHEMICAL NATURE: A dramatic difference was reported in the base composition of seed DNA from 4B and 0B strains of maize, not supported by later data on base composition of leaf DNA. By contrast, DNA of two B chromosomes containing populations of the grasshopper *Myrmeleotettix maculatus*, contains an appreciable quantity of a satellite DNA with a lower cytosine and guanine content than the main DNA fraction, which is not present in 0B populations. In Britain, populations contain two structural types of B chromosomes, metacentric and submetacentric, particularly in warm dry environments. They are scarce or absent in humid, cooler localities and completely lacking in European populations. This satellite apparently differs amongst populations and is not homologous to main band DNA. The DNA of B chromosomes, from a study of maize seedlings with and without B, through buoyant density distribution at CsCl gradients, has been concluded to be very closely related to that of A chromosomes. How far this relationship is present in other plants as well, has not yet been worked out.

In rye, the presence of B is related to an increase in histone but decrease in total protein content. The number also appears to have an effect on total protein content, as seen in maize. The amount of repetitive DNA seems to be involved as well. In a particular population of grasshopper with 28% highly repetitive DNA, the B chromosomes would not pair with those of other populations. In maize, on the other hand, no relationship could be recorded between B chromosomes and repetitive DNA.

EFFECT ON POPULATIONS: The common property of accessory chromosomes is their deleterious action or in certain cases, their lack of activity (Müntzing, A 1967 *Genetics—basic and applied* LTS Forlag Stockholm). In plants where they exert a positively harmful effect, as rye, they are retained in the population only through their property of doubling their number by non-disjunction. In *Festuca pratensis*, *Lilium medeoloides* and *Centaurea scabiosa*, however, evidences indicate that when present in low numbers under certain circumstances they may exert a favourable effect on plant vigour. B chromosomes have been shown in certain cases to modify chromosome pairing in hybrids between closely related species, restricting them to exact homologues, as seen in *Lolium*, wheat and fescue. However, the effect was not observed at the tetraploid level and even at the diploid level, the involvement of chiasma frequency was not clear.

ORIGIN: The origin of accessory chromosomes is rather obscure. They may have been derived from the ordinary chromosomes sometime in the remote past, starting possibly as heterochromatic centric fragments, which through chromosomal rearrangements, acquired their present nature. Any of the processes leading to the loss of chromosome material, as given in earlier chapters, could have led to the formation of B chromosomes, According to White (1973), they can be regarded as a special type of genetic polymorphism not subject to Mendelian inheritance due to special accumulation mechanisms. He regards the supernumerary chromosome segments inserted in or attached to the normal chromosomes, recorded in many grasshoppers and other animals, as somewhat analogous to accessory chromosomes. These, however, obey the ordinary Mendelian laws. They are usually heterochromatic, arising through duplication or deletion of the heterochromatic segments originally present in the karyotype. An example is the Australian grasshopper *Austraicetes interioris* showing two types of chromosomal polymorphism. It is polymorphic for pericentric inversions in three pairs of autosomes; but the mutually inverted segments differ in the size and distribution of the heterochromatin contained in them.

Darlington CD (1950) first suggested that since accessory chromosomes have been recorded more frequently in diploids than in the polyploids, they may be responsible for imparting variability to a species and hence their presence in polyploids is no longer of any significance. In *Allium stracheyii* ($2n=16$), diploid individuals collected from temperate areas of the Eastern Himalayas showed 1-10 B chromosomes. However, on growing them in the plains, under tropical conditions, the plants became polyploids and the B chromosomes disappeared. The conversion to polyploidy was apparently due to temperature shock. Such loss of B chromosomes can be explained by either of the following two ways: (i) the B's, on being brought to the plains, degenerate because they are ineffective and the degeneration-products in the cytoplasm disturb the spindle mechanism, leading to polyploidy, or, (ii) polyploidy is caused by temperature shock. In these cells the B chromosomes, due to their redundancy, degenerate or fail to survive in competition with the increased number of normal chromosomes for availability of constituents required for metabolism (Sharma AK and Aiyangar HR 1961 *Chromosoma* **12**: 310).

This evidence suggests that an increase in chromosome constitution may not be always beneficial to the individual. In such cases, a gradual loss of function of some of the extra chromosomes and subsequent heterochromatinisation may lead to the origin of accessory chromosomes.

The disappearance of accessory chromosomes through successive cell cycles in induced polyploids indicates their redundance, in terms of the better adaptability of polyploids due to doubling of gene dosage. The diminution in chromosome size as normally observed in polyploids—which has been earlier explained on the basis of the condensation of functionally redundant heterochromatic segments—is a phenomenon similar to the loss of B chromosomes with the induction of polyploidy.

## Conclusion

The concept of B chromosome as being heterochromatic in nature has led to the understanding that these chromosomes are highly variable in structure and behaviour. Their nature and the effects of their presence can be explained more plausibly taking into account the presence of repetitive DNA in most of them. Repetitive DNA is regarded to be present in clusters in chromosomes. B chromosome may be the ultimate effect of this clustering into an entire chromosome.

CHAPTER V: 7

# DETECTION OF BASIC NUMBER AND AFFINITIES OF SPECIES

In the majority of angiosperms, the chromosome number often provides a clue in determining the status of a species. Usually a low chromosome number indicates primitiveness. A higher number suggests that it may be derived from a lower basic one in the course of evolution. Both cytological and genetical methods are available for determining the basic number of a species, its degree and type of polyploidy and its affinities. The principal cytological methods include both meiotic and mitotic studies.

MEIOTIC ANALYSIS AND SECONDARY ASSOCIATION: In the analysis of meiosis, alterations in chromosome behaviour indicate absence of homology.

(i) The formation of regular bivalents without any aberrations indicates the true diploid nature of the species and its comparatively primitive status.

(ii) Multivalent formation suggests the presence of polyploidy of different degrees. A regular number of multivalents formed by all chromosomes is due to duplication or multiplication of the entire genome, showing autopolyploidy. Irregular multivalents involving only one or more chromosomes suggest its trisomic or polysomic constitution.

(iii) Secondary association of bivalents is a phenomenon observed during the meiosis of plants, in which bivalents are associated in groups of different numbers at diakinesis and first metaphase, even persisting in some cases up to second metaphase. It can be used in determining the basic number of a species. It may be defined as the association or grouping between bivalents generally observed at diakinesis and metaphase I, indicating a distant homology amongst the members of the group not sufficient to ensure pairing. Darlington first observed this association in *Prunus* and used it to determine the basic number followed by other authors. This phenomenon is supposed to be due to the association of ancestrally homologous chromosomes which have been separated from each other in the course of evolution, have undergone structural changes and have finally got the chance of meeting again within the same zygote. They cannot pair completely due to the evolutionary changes they have undergone during the period of separation. Since the formation of bivalents suggests duplication of the chromosomal set, its occurrence shows that amphidiploidy has taken place.

In counting the basic number from secondary association, two types of counts have been suggested—(a) the association that occurs in the maximum frequency, or (b) the minimum number of groups present showing maximum association. The latter method is correct because the homology between the bivalents is not enough to overcome all the intracellular barriers which lie between their association in all cases. Therefore, wherever the minimum grouping occurs, irrespective of its frequency, it may be taken to represent the basic number.

Objections have been raised to the counting of the basic number from secondary association by several workers who regarded the phenomenon as an artefact out of the effect of fixatives. It has been interpreted as a pattern arising out of unequal forces of repulsion within the nucleus, leading to association without any significance. This idea is invalidated by the association of chromosomes of different sizes having distant homology. Thomas and Revell suggested that it arises out of random fusion of heterochromatic regions in early stages due to their sticky nature. In later stages these result in highly stretched interbivalent connections and groupings which are non-specific and without any significance. Sharma AK (1954 *Genetica* **27**: 323) found secondary association and interbivalent connections in a number of genera, including Commelinaceae arising due to fusion of heterochromatin. But these fusions are strictly specific occurring between related bivalents only. In the minimum grouping or maximum association, no such connections can be seen between members of different associating groups. The genetically more active euchromatic regions of homologous chromosomes undergo structural changes during evolution to such an extent that they lose all homology. Heterochromatic regions however, being comparatively inert, undergo much less structural changes and therefore, when these once homologous bivalents meet again, the heterochromatic regions are still capable of pairing in a modified way in the form of fusion, a process enhanced by their sticky nature. The occurrence of repetitive DNA in these regions supports this contention that in spite of alterations during evolution, certain genes retain their ancestral characters. Therefore on the basis of these findings, secondary association aids in finding out the phylogeny of amphidiploids.

This method has been utilised in determining the basic number of cultivated rice, *Oryza sativa* ($2n=24$). It shows a maximum association of two groups of three and three groups of two bivalents, the basic number being deduced to be $x=5$. It probably arose from an ancestral species with $n=5$ chromosomes (ABCDE). Structural alterations and duplication of one chromosome led to a new complement in the course of evolution $n=6$ ($A_1B_1C_1D_1E_1E_1$). Chromosome duplication of another chromosome in the ancestral set gave rise to $n=6$ (AABCDE). Hybridisation between these two forms, followed by a duplication of the entire genome resulted in the present amphidiploid cultivated rice with $2n=24$ ($A_1A_1B_1B_1C_1C_1D_1D_1E_1E_1E_1E_1$, AAAA BBCC DDEE). The 12 bivalents associate in first meiotic division to give $2(3)+3(2)=5$. Analysis of the karyotype shows roughly five groups of chromosomes.

(iv) Formation of rings during meiosis indicates the genomic constitution of the species. A multivalent ring shows the polyploid composition, isochromosome ring the presence of an isochromosome and a translocation ring with alternate segregation that the individual is a translocation heterozygote. A large bivalent may also form a ring.

(v) The presence of inversion bridges with fragments denotes an inversion heterozygote. Univalents are caused by a hybrid constitution of numerical nature.

(vi) Heteromorphic bivalents represent structural heterozygosity, as seen in *Cipura paludosa*. Other meiotic abnormalities like non-disjunction of bivalents, lagging and early separation represent lack of co-ordination between the chromosomes and the spindle. The entrance of a particular genomic set into foreign cytoplasm leads to such effects, which indicate that the individual is not a true diploid.

PACHYTENE STUDY: Study of the pachytene chromosomes, wherever feasible, is an excellent method for detecting minor changes like small inversions or terminal deficiencies, otherwise undetectable in meiotic or mitotic chromosomes.

KARYOTYPE STUDY: Comparison of karyotypes of species shows their interrelationship and phylogeny and indicates the lines of evolution. This aspect has been discussed in detail in another section. The advancement in banding pattern technology has rendered the comparison much more precise and future observations are expected to yield data of substantial importance in elucidating the aspects as yet unknown.

STUDY OF THE NUCLEOLI: A true diploid species is expected to possess two nucleolar chromosomes and therefore two nucleoli of identical dimensions at telophase. A heteromorphic pair of nucleoli formed by an apparently identical pair of nucleolar chromosomes is indicative of structural differences between the two chromosomes as in *Scilla peruviana*. Duplication of nucleoli usually takes place through duplication in the number of the chromosomes concerned. However segmental interchanges between nucleolar and non-nucleolar chromosomes may also be responsible for an increase in the number of nucleoli within a diploid species, as observed in *Galtonia*.

GENETICAL METHODS: Genetical methods include hybridisation and have been used widely in crop plants. They are based on the principle that compatibility between species is directly proportional to their degree of affinity.

CHAPTER V: 8

# CHROMOSOMES IN EVOLUTION

## Role of chromosomal rearrangements

The structure and the many-faceted behaviour of chromosomes represent a level of organisation above that of DNA biochemistry. A further level is indicated by the cytogenetic structure of natural populations and a still higher one by the evolution of species. The natural incidence rate of structural alterations is more than adequate to produce the natural variations, though most of them are lost in the course of evolution. Some might appear as random, floating changes while others may develop into adaptive polymorphisms within species, operating usually at the heterozygous state. The changes which might result in speciation are expected to be homozygous. However, some plant groups are known to preserve a uniform karyotype, like the bimodal karyotype in the Aloineae. At the other extreme, the genus *Crocus* shows extreme variations in the karyotype (see Jones K 1978 *Bot Rev* 6: 120) and almost each and every species can be distinguished by its chromosomes. The reasons for these very different degrees of control are not yet clear.

Evolution is essentially a cytogenetic process and ultimately, a complex sequence of changes in the chromosomal DNA. For a full understanding of genetic systems in evolution, both population genetics and cytology are essential. Chromosomal mechanism itself varies in a great many ways between different taxa, showing that it has undergone an evolution of its own, which underlies the visible evolution of phenotypes. The role of chromosomal rearrangements is two-fold: (i) in generating cytogenetic polymorphism, principally through the establishment of heterozygote superiority, and (ii) in the origin of new species through genetic isolation from the development of non-viable heterozygotes. Phenomena allied to chromosomal rearrangements are the evolutionary increase and decrease in chromosomal DNA, involving on one hand duplication of genetic material and on the other hand, its loss. There are other aspects as well, which are unique in occurrence and present facets which must have affected evolution profoundly but their role is yet not fully understood. Notable amongst them are the different forms of accessory chromosomes, the aberrant divisional cycles observed in several groups, the processes of heterochromatinisation and dosage compensation, as also sex determination. Evolution is definitely the projection, into the geological time scale, of the processes of mutation, genetic recombination and natural selection as they are going on commonly and continually in all populations of organisms. But in every stage of this process, unique events and their combinations occur as well. Chromosomal changes involve a series of gene changes, which, though unique for the event, can rearrange and reassort themselves in a series of new combinations during evolution.

POLYMORPHISM AND SPECIATION: In compiling the available data regarding the relative roles of chromosomal rearrangements in polymorphism and speciation, a parallel is observed to a certain extent. For example, paracentric inversions are the commonest forms of chromosomal changes occurring as polymorphisms in populations and also between the karyotypes of different species of *Drosophila*, similar to pericentric inversions in trimerotropine grasshoppers. However, many other cases are known where particular types of chromosomal alterations, frequent in the evolution of species, do not survive as balanced polymorphisms. A well known case is that of morabine grasshoppers. In the phylogeny of this group, 60 or more fusions and dissociations have been recorded but none of them exist as polymorphisms within a population. This observation can be explained through the basic contrast between balanced polymorphism and speciation, since the former results from cohesive forces and the latter from divisive forces in natural populations.

When chromosomal rearrangements observed both within a species and between different species exhibit a similar trend in a group, balanced chromosomal polymorphism might have led to cytotaxonomic differences at an interspecific level. A single chromosome sequence may be fixed for an incipient species and an alternative one for another species. Alternatively, one species may maintain both sequences and the other one may adopt only one. The transformation of a polymorphic population into one or more incipient species, each with a single chromosome sequence, may be assisted by the new species occupying different ecological niches, to which they are better adapted.

In certain groups, on the other hand, fusions and dissociations which exist as interspecific differences have not been preceded by a state of balanced polymorphism in the ancestral population. Speciation in such cases has not been affected through an accumulation of balanced polymorphisms; though chromosomal changes have obviously been effective. The rearrangements, however, must not reduce the fertility of the heterozygotes too drastically to be effective in the formation of a species. For example, tandem fusions are few and far between in such species in spite of the fact that the theoretical possibility of their occurrence is the same as centric fusions and dissociations.

## Different aspects of chromosomal evolution

In the evolution of animals, John and Lewis (1965) have allotted a major role to mutual translocations (other than dissociations and fusions), with particular emphasis on acrocentric chromosomes. White (1973), on the other hand, through a critical study of the different groups, suggests that this viewpoint is contrary to all principles of mathematical genetics and is contradicted by an analysis of the polytene chromosomes of the Diptera.

A fundamental feature of chromosomal evolution is the quantitative one, involving the extent of alteration the basic haploid amount of DNA in the karyotype has undergone during speciation. The associated phenomena are increase in DNA through duplication, inactivation through heterochromatinisation and its loss through the deletion of heterochromatin blocks. The observations indicate that fully active genetic loci can be converted by mutation into partially or completely inactivated heterochromatic ones. Whether the reverse process, that of the conversion of inactivated loci into fully active ones, occurs, is not yet known. An associated phenomenon is decrease in chromosome size with polyploidy, without involving a decrease in DNA content, as observed in a number of plants. It has been explained through the condensation of heterochromatic segments in the polyploid level due to their redundancy (Sharma 1970).

Another aspect of chromosomal evolution deals with the changes occurring in the total amount of genetic recombination. The range is a wide one, from $n=1$ or 2 in *Parascaris equorum* to about $n=220$ in *Lysandra atlantica*. According to one view, natural selection controls the amount of genetic recombination both between individual polymorphic loci and the total amount in the entire karyotype. Thus, the amount of recombination between polymorphic nuclei is being continually directed by selection while the type of life cycle population structure or environment is the major factor controlling the total genetic recombination in a genetic system. Recombinations in particular chromosome segments may be increased through artificial selection, but possibly not of the entire karyotype. Proponents of the opposite viewpoint suggest that recombination depends on many cellular processes. Its amount may be affected by selection for those chiasma frequency and distribution which lead to meiotic efficiency. Both these viewpoints may be correct to a certain extent.

## DNA changes in differentiation and evolution

The lack of apparent nuclear activity, as shown in the absence of chromosome division in differentiated or adult tissue, posed a problem since the process of differentiation involves vigorous gene action. Endomitosis was suggested by earlier workers like Geitler and Huskins to account for this phenomenon. Endomitosis or endopolyploid reduplication visualises a geometric increase in the number of chromosome threads *without* consequent nuclear division. Induction of division in the adult tissue, through nucleic acid precursors and even individual sugar moiety by other workers including the author, showed the presence of endopolyploid nuclei as well as diploid ones, proving that nuclei are fully active in such tissues.

Since differentiation is a continuous process, uninterrupted transcription is required. The limited transcribing life of a DNA molecule needs a continuous supply of fresh DNA strands. For this process endomitotic replication has been suggested to take place at regular intervals (Sharma AK 1976 *Proc Ind Nat Sci Acad B* **42**: 12). In endomitotic nuclei, the amount of radioactive uridine uptake is the same as that of diploid nuclei, suggesting that only one strand is active in transcription. Dupli- cation of the DNA strand leads to gradual polyteny without however the corresponding cell division, which would have resulted in loss of symmetry and equilibrium through uncontrolled tissue growth.

DNA endoreduplication and related phenomena, such as endomitosis, polyteny, nuclear restitution and somatic polyploidy are widespread in the plant and animal kingdoms, though most frequent amongst insects and angiosperms. However, some species do not show endopolyploidy and the phenomenon appears to be related to the basic nuclear DNA content. It seems, therefore, that the differentiation and specialised func- tions of different tissues of eukaryotic organisms require a defined mass of DNA to maintain their functional and regula- tory state. The requisite amount of DNA may be achieved by (a) tandem duplication of DNA sequences (*saltatory replication*) leading to the increase of predominantly repetitive DNA families or (b) generative or germ line polyploidy or (c) increase in the tandem segments in polyploid chromosomal sets, leading to larger chromosomes but maintaining a stable number (*crypto-polyploidy*). According to Nagl (W 1983 In: *Proc II Kew chromosome conference* George Allen and Unwin, London), when the basic nuclear DNA is not increased phylogenetically by either of these methods, an alternative evolutionary strategy is adopted by the taxon involving DNA endoreduplication, polyteny and somatic polyploidy.

## Heredity and evolution

In a final analysis, nothing makes sense in biology except in the light of evolution. It is, in a way, the converse of heredity. Heredity tends to make the progeny like their parents. At the molecular level, a precise replication of the DNA code ensures the continuity of the gene, and the character it controls, from generation to generation. Heredity is thus a conservative force. Evolutionary innovation demands that it be occasionally thwarted. This is what results when mutations and at a higher level of organisation, chromosomal changes take place. Mutation, recombination, selection and isolation, at the preliminary level, form the four corner-stones of evolution (Mayr 1969). Genes specially favoured by selection either produce heterozygotes of high viability or produce viable combinations of the greatest number of genetic backgrounds. The mere change of the genetic environment may change the selective value of a gene very considerably. Such gene changes—individually and collectively—form the raw materials utilised by natural selection to provide the requisite evolutionary lines. The new genotypes are established within a population, aided by two main processes, natural selection and genetic drift. Of them, natural selection is the more important agent. It is relatively efficient in increasing the number of advantageous dominant alleles and this also applies to recessive alleles, when present in higher frequencies. Genetic drift aids in the establishment of non-adaptive, neutral characters and even slightly unfavourable traits.

## Evolution

Natural selection has certain obvious limitations. It can only produce results which are of immediate biological utility to the species and being blind and automatic, is incapable of purposeful design or foresighted planning. In consequence, its results will always be relative to the particular environment in which the particular species is living as well as to its particular structure and habit (see Huxley J 1954 The evolutionary process in *Evolution as a process*, George Allen & Unwin).

Genes with either or both of the following two properties will be specially favoured by selection.

i) those producing heterozygotes of high viability, and

ii) those producing viable combinations on the greatest number of different genetic backgrounds. The mere change of the genetic environment may change the selective value of a gene very considerably.

EVOLUTION OF MAN: In the evolution of man from his closest ancestors, possibly gene mutations have been more effective than chromosomal changes. This process has been a slow and complex one. Long continued directional selections, replacing older genes by new, gradually transformed the pre-human organism to a genetic complement on which human culture arose and developed. The human traits were favoured since they conferred certain advantages in their transmission to the succeeding generations in a higher frequency. Mutation and recombination, followed by selection and drift, have been active in the later origin of racial diversity. Inbreeding of the already diverse populations further led to new types of gene pools. This continuous, sometimes slow but unfailing gene flow observed between neighbouring clans, tribes, nations and races upholds the biological and evolutionary unity of mankind as a single species.

BIOLOGICAL AND CULTURAL EVOLUTION: Insofar as man is concerned, he is engaged simultaneously in two kinds of evolutionary development—biological and cultural (Dobzhansky TH 1964 *Heredity and the nature of man* George Allen Unwin, London). His biological heredity is transmitted very much as in the other organisms. His culture is, however, not carried on by genes in the sex cells but acquired anew by each individual from his surroundings. Like his genes, his cultures have also evolved and are still evolving. The rate of biological evolution is very slow. For example, assuming that the human karyotype contains some five billion nucleotide pairs, differences accumulate at the rate of some five nucleotide substitutions per year. An ever increasing rate, on the other hand, characterises cultural evolution and it has also affected biological evolution to a large extent, neutralising or modifying the effects of natural selection in many cases. Though the adaptation by culture is enormously more rapid than genetic adaptation, yet the two processes are not alternative or mutually exclusive. They are connected by a feedback relationship, inasmuch as in man, genes stimulate the development of culture. The cultural improvements, in turn, activate genetic changes which facilitate the further development of culture. The normalising and balancing forms of natural selection, which serve to weed out the harmful genes, have become relaxed in human populations through remedial measures conferring greater longevity. In planning human evolution therefore, biology must be guided by man's cultural and spiritual heritage as well as considerations of his physical and mental well-being.

An alternative theory of orthogenesis indicates that evolution follows a straight line or it is directed by some force residing within the organism itself. Teilhard de Chardin (1959) has postulated a philosophical or mystical agency in the progressive evolution of mankind leading to an ultimate convergence of all separate consciousness in a harmonious union at the "Point Omega". In the Hindu philosophy, the evolution of all forms of life is through a series of incarnations, from the lowest through all the intermediate stages to man and finally the evolution of human consciousness to submerge into the supreme all pervading consciousness. The chasm between human evolution as visualised by philosophers and as has taken place at the physical level, through many millions of gene changes, over many millenia, remains to be bridged.

Human cultural evolution has not been an unmitigated blessing. It has also affected the environment, particularly after the industrial revolution and the wars—world and regional—by releasing chemicals and radiation from various sources over a very short period of time. Against the background of the long periods of gene-environment interaction during adaptation and evolution, this simultaneous attack on the genetic complement by various agents, internal and external, may upset the evolutionary balance. The consequences are unpredictable and therefore, in the interest of all living organisms, augmentation of the environmental pollutants—accidental, experimental or otherwise—should cease.

# SECTION VI
# EPILOGUE

CHAPTER VI: 1

# PRESENT CONCEPT OF CHROMOSOME

AN attempt has been made in the following pages to summarise the present ideas. For details, the reader is requested to read the previous chapters of this book.

## I. EVOLUTION OF THE EUKARYOTIC CHROMOSOME

The complex eukaryotic chromosome has evolved through a series of steps from the simple prokaryotic genophore—a naked DNA thread. A possible intermediate stage is the chromosome of Dinophyceae. It has the definite nuclear membrane and well defined structure characteristic of the eukaryote chromosome but differs fromthe latter in the almost absence of histones and of a mitotic mechanism. In the course of development of the mitotic apparatus, certain genes were allotted the function of spindle formation. Possibly at first they were diffuse, as in the Conjugales. Later the performance was localised in a specialised region, the spindle organiser or centromere. Thus, in the course of evolution, the function performed by a large number of genes was relegated to a block of genes localised in a specialised segment (Sharma AK 1969 *Nucleus* **12**: 86).

Similarly the patterns presented by the heterochromatic segments in different taxa may indicate a primitiveness or advancement with regard to certain functional segments of chromosomes, irrespective of the state represented at other loci. The secondary constriction regions are examples of such grouping of genes with a similar function In *Musa* and *Scilla*, for example, a large number of secondary constrictions with a corresponding number of nucleolar organisers have been observed. Further localisation of the property of nucleolar organisation has resulted in fewer numbers of chromosomes with secondary constrictions, as observed in the majority of angiospermous genera.

## Evolution in chemical composition of the chromosome

The complexity of a eukaryotic chromosome can be realised from the observation that a human haploid cell contains chromatin matter about one meter in length. The multifarious activities performed by the chromosomes are made possible by this complexity in their structure. They have to control the diverse requirements of cell metabolism and sequential and phasic cellular growth at the same time as cellular differentiation. They perform mutually incompatible functions, including recombination and mutation, which aid in the origin of new forms, and regular cell division, which maintains stability. Certain genes have specific assignments while other vital activities, like replication of the same genes, are controlled by other genes.

These diverse functions performed by the same chromosomes depend on the diversity of their chemical make-up and the dynamism exhibited in their behaviour (Sharma AK 1976 *Proc Ind Nat Sci Acad B* Silver Jubilee Lecture). This concept of dynamism explains the chromosomal control of regulation, differentiation and reproduction in the eukaryotic systems. The chromosomes contain—in addition to DNA—RNA histones and non-histones, as well as divalent cations. These components have been isolated and analysed in different mammalian systems. Protein is regarded to aid the condensation of chromosomes, control of DNA and RNA synthesis and maintenance of chromosomal integrity.

Histones are mostly associated with the condensed state of the chromosomes. They are, however, observed also in the dispersed state in polytene chromosomes. In the activation of transcription, the DNA-histone link may be loosened, rather than the complete removal of histone. Since the histones do not have the extent of diversity required to control the large variety of characters involved, the non-histone proteins may have an important role in this connection.

The increasing complexity of the eukaryotic chromosome structure is accompanied by a corresponding complexity of the DNA skeleton through the diversification of repeated, highly repeated and non-repeated unique sequences. Such repetitive sequences are very common at all levels of the eukaryotic system. Satellite DNA has a definite role in ribosomal RNA synthesis and nucleolar metabolism as shown by molecular hybridisation. Repeated sequences are very common in plants as well. About 70-75% of molecular DNA in rye is made up of repeated sequences.

One of the most interesting findings in recent years is that the majority of the genes in nucleated cells are fragmented, as also are those of viruses that invade the nuclei of such cells. Bacterial genes, on the other hand, are not fragmented. The number of fragments is quite high, being 52 pieces in the alpha-collagen gene. The stages in the expression of these genes are given in Fig. VI:1.1 (from Lewis R 1981 *Science* **212**: 28).

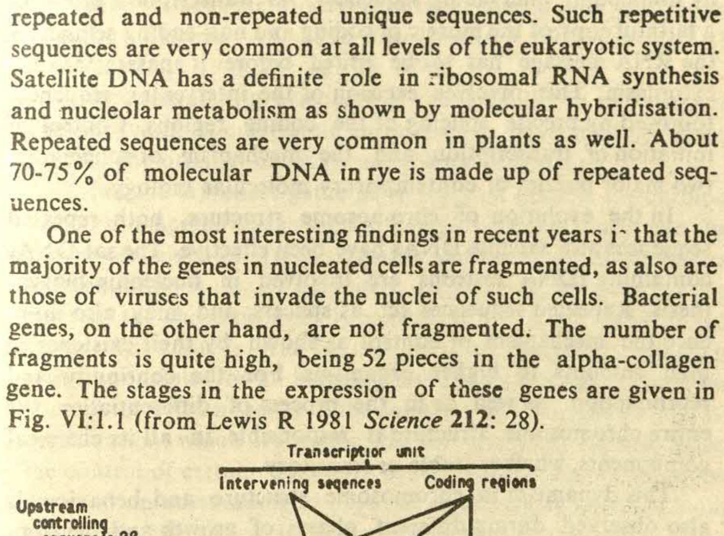
Transcription unit
Intervening seqences
Coding regions
Upstream controlling sequences ??
CCAT TATA
5'
3'
DNA in chromosome
-80 -30
Promoter region
Cap
Poly A
Transcription
Cap
Poly A tail
RNA transcript
Intervening sequences are degraded but they may serve as signals to turn on or off other genes
?
RNA processing – which takes place on unknown structures in the nucleus – removes intervening sequences and splices coding regions
Mature messenger RNA
Goes to cytoplasm where code is translated into protein sequence

**Fig. VI: 1.1**

### Stages in expression of genes from higher organisms

Genetic information encoded in the DNA of the chromosome is first transcribed into an RNA copy which then forms the template for translation into the amino acid sequence of a protein. In contrast with bacterial genes, most genes in higher organisms are fragmented as discrete coding regions separated by non-coding intervening sequences. As transcription produces a faithful copy of the mosaic of coding and non-coding sequences, the RNA message has to be edited before it passes into the cytoplasm. This involves excision of the intervening sequences followed by precise splicing of the coding regions. Control of initiation of transcription and the mechanism of splicing are two major puzzles of contemporary molecular biology.

In the evolution of chromosome structure, both repeated sequences and satellite DNAs have been effective. The sat-DNAs containing rDNA cistrons are involved in nucleolus biosynthesis. Repeated sequences act as spacers, and may also influence the mechanism of control as shown by their existence in high amounts in higher organisms. For the continuity and reproduction, as well as in the process of differentiation, the entire chromosome structure is responsible, in all its chemical components, whether stable or transitory.

This dynamism in chromosome structure and behaviour is also observed during different phases of growth and development (Sharma AK 1978 *Proc Ind Acad Sci* **87B**: 161). The ratio of DNA varies from organ to organ as also the proteins. Evidences are being gathered to indicate that while maintaining a basic genetic skeleton, eukaryotic chromosomes change the patterns of their constituents during development and differentiation for the regulation of all vital activities. The switchover from mitosis to meiosis in the formation of the germ cells and further reversion to the mitotic behaviour of the generative nucleus are further examples of dynamism. The occurrence of endoreduplication in differentiated tissue indicates that replication of a chromosome becomes necessary when the transcribing capacity of a single DNA strand has been exhausted. This altered method of replication fulfils the need of obtaining a fresh strand of DNA without adding to the number of cells essential for symmetrical growth. Data along these lines have been obtained from the author's and other laboratories (Nagl W 1976 *Nature* **261** : 614). The inconstancy in the chromosome complement observed in the somatic cells of vegetatively reproducing plants, as discussed in earlier chapters, plays a significant role in the origin of new forms through their participation in the formation of daughter shoots. This mechanism gives the species an effective tool to secure new genotypes for speciation without the process of sexual reproduction and recombination (Sharma AK 1956 *Caryologia* **9** : 93; Sharma AK and Sharma A 1959 *Bot Rev* **25**: 574).

The accumulating evidences of the flexibility in the structure and behaviour of eukaryotic chromosomes have modified considerably the original chromosome theory of heredity.

From the evidences gathered in the past decade, the chromosome has emerged as the highest complex organic molecule that can be conceived of. In this gigantic molecular set up functional differentiation is clearly delimited. Replication, an essential property of the chromosomes, is controlled by certain genes in this set up. The same is true for cell division. Similarly, transcription responsible for all heterocatalytic action is again under the control of certain genes in the same set up. Mutability and recombination, which are responsible for variability are also under the control of certain genes. In other words, the chromosome serves as an outstanding example of an extremely complex living structure where the constituent units themselves control their own functioning and their own metabolism and even their own reproduction (Sharma AK 1983 In: *Proc II Kew chromosome conference* 35, ed Brandham PE and Bennett MD, George Allen and Unwin, London).

In order to exert this control, the property of changing the structural pattern of chromosomes, both physical and chemical, while maintaining the stability of the genetic material had to be achieved. Simultaneously, a flexibility allowing alteration in their behaviour to meet the demands of sequential growth, development and reproduction in eukaryotic systems became inevitable. This emergence of complexity on the other hand, and dynamism in structure and behaviour on the other, have been the principal features of evolution of chromosomes from lower to higher forms of life.

This very dynamic property allows the chromosomes to undergo variations in their behaviour and structure—both physical and chemical, in different organs, in nuclei at different stages of differentiation, both meristematic and adult, and under varied conditions of reproduction—both sexual and asexual. This inherent plasticity is responsible for the tolerance of chromosomes to genetic engineering, viable reshuffling of chromosome segments and adaptation to altered environment—both intra and extracellular. The optimism expressed in recent years of the possibility of the insertion of chromosomes or genes in the cell and of the inserted genetic material being adapted to the cellular control system depends entirely on the exploitation of this dynamic and flexible property of chromosomes, through further refinement of molecular and cellular methodology (see Sharma AK 1978 *Proc Ind Acad Sci* **87B**: 161; 1981 *The Nucleus* **24** : 87; 1984 *Proc Indian natn Sci Acad* **50B**: 1).

## II. ADDITIONAL GENETIC ELEMENTS IN CHROMOSOMES*

### A. Repeated DNA

Chromosomes of higher plants have been shown to contain thousands of similar nucleotide sequences of different categories as well. The amount of heterochromatin rich in repetitive sequences varies extensively between related species. In majority of cases, the arrangement of satellite DNA or large homogeneous repeats at major segments and small repeated sequences interspersed throughout the chromosome has been noted. Such DNA repeats have been found also in centromeres, secondary constrictions and telomeres.

Why are such high degrees of repeats present in chromosomes and what is their function? The role of satellite DNA in the formation of ribosomal RNA has been established through molecular hybridisation technique but it does not account for the entire amount of satellite DNA present. Such DNA has often been termed as *trivial DNA* accumulating in the genome during evolution by gene duplication though in the mammalian system it has been shown to compose nearly 95% of the total DNA. Species with large amounts of heterochromatin have more total DNA than species with low heterochromatin content.

The regulatory role of repeats has been suggested by various authors. A chromosome structure has been visualised as a linear array of potentially independent replicons terminated by short

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*Sharma AK (1978) *Nucleus* 21 : 113; Sharma AK (1984) Presidential Addresses, Golden Jubilee Publication, Indian National Science Academy, New Delhi.

palindromic or inverted repeat sequences; a structure with major repeats at one locus and minor repeats interspersed throughout, for control of unique sequences, for allowing flexibility in functioning of uniques, and for accumulation of mutations. The role of repeats as spacers amongst unique sequences has been noted in 5S RNA sequences. Even their role as protector of vital genes and kinetochores against radiation and other injury has been claimed. Localisation of the major homogenous repeats at one locus has been regarded to be advantageous for the chromosome as it would not interfere with normal functioning.

Acquisition of reiterated sequences is a feature of evolution in higher plants. The B chromosomes in plants show a high degree of repeats, their addition leading to an increase of almost 4% DNA. Reviewing the data that the plant system possesses multiple copies of DNA sequences in different segments, repeated DNA in B chromosomes as well as chromosomes which have no apparent phenotypic effect, Sharma (1978) suggested that these additive genetic elements may serve as regulators, spacers or act as loci for the accumulation of mutations.

### B. Dynamic DNA

Since the recognition of repeated sequences in DNA, additional genetic elements have been variously interpreted. Different terms have been assigned to them on the basis of their property, sequence complexity and location, but none of the epithets applied to such DNA does justice to the multifaceted nature of its composition and function.

*1) Unique genotypic DNA* coding principally for structural proteins, constitutes a fraction of the total amount of DNA per cell within a eukaryote. Such DNA is present in a single copy, or in a few copies in certain cases, such as in α-globin or β-like globin in mammals. Single copy DNA, with no apparent function, as in *Xenopus*, is an exception.

The other types of sequences, so far identified, are repeats, nucleotypic, intervening, selfish, symbiotic, parasitic, ignorant, incidental and pseudogenes or dead sequences. The esoteric DNA is excluded from this discussion since it refers principally to mitochondria.

An analysis of these epithets and the parameters involved, indicates that they are diverse manifestations of a special class of DNA, which can more appropriately be designated a *dynamic DNA*. The rationale behind the adoption of this term is based on certain evidences, derived from the different types of DNA described earlier. The different categories of almost non-unique sequences are outlined below.

*2) Repetitive DNA:* The reiterated sequences may be highly homogeneous, like satellite, or moderate, middle, minor and inverted or palindromic. The repeats may be coding or non-coding in nature.

Of the transcribable sequences, those coding for histones, tRNA and rRNA, are derived from repeated sequences of varying order, in different organisms, through localised amplification. They may exhibit a high degree of polymorphism. The regulatory or controlling function has been ascribed to moderate and minor repeats, interspersed between the unique sequences. Moderate repetitive DNA has been suggested to influence evolution and "adaptive radiation". Diverse repeats in specific chromosomes have been reported at least in Drosophila and Man. Intermediate repeats were observed in different positions on chromosomes in different strains of Drosophila. The reasons for these differences have not yet been established. It is quite likely that their diversity may indicate a functional basis.

Some of the repeated sequences may have a regulatory role on chromosome structure, on its folding or pairing in the differentiation of centromere, heterochromatin and spacers. Such sequences in rye heterochromatin exhibit rearrangements, each simple subrepeat being interspersed by unrelated non-subrepeats. Other non-coding repeats, arising also through localised amplification, may show polymorphism in different organs. They have been claimed to exert control on evolution as well. They have also been suggested to serve as loci for accumulation of mutations or as a reservoir of new sequences. These ideas further support Ohno's earlier suggestion of evolution by gene duplication.

*3) Intervening sequences*, or *introns* in eukaryotes cause intragenic interruptions which led to the split gene concept. They may be compared with insertion elements. These molecules may occur in multiple copies, are transcribed but are then excised and remain untranslated. Their location in intercalary positions and at termination and initiation points of structural sequences may indicate their role in processing of mRNA, transport and translation.

*4) Transposable elements:* Eukaryotic sequences which could be transposed, affecting gene expression, were initially recorded by McClintock in maize. Similar elements with adjacent high degree of repeats but without any detectable functions were later observed, such as Ty-1 in yeast and 412 Copia and 297 of Drosophila. Such sequences and their products aid mobility, survival and promotion of genetic diversity. In yeast, such middle repeats are present in 35 dispersed copies, constituting almost 2% of the genome.

*5) Selfish DNA* is a term applied to DNA sequences, existing in the cells entirely due to their capacity for replication and survival in a congenial cellular environment without any positive evolutionary function. Their existence may not be harmful to the phenotype, like the segregation-distorter locus in Drosophila. Nearly 30% of the DNA in eukaryotic cell may exhibit this property. Orgel and Crick had suggested that this DNA may include even middle repeats, introns and intergenic sequences. Qualitative and quantitative changes of such middle repeats may influence generation time and cell size.

*6) Parasitic* and *7) Symbiotic DNA:* Parasitic DNA can be regarded as a synonym for selfish DNA to a certain extent. It includes such amplified segments as are capable of movement between different chromosomes. The gradual acquisition of useful properties makes them symbiotic in nature.

*8) Ignorant DNA* is comparable to selfish DNA with respect to amplification and dispersion. Its origin is sequence-independent as compared to selfish DNA, which is sequence-dependent. This definition implies that transposons like Copia, Ty-l, are selfish while ignorant DNA involves the sharing of similar sequence patterns between chromosomes.

*9) Incidental DNA* is the term applied to selfish DNA sequences, which have arisen due to high rate of mutations, to fulfil the need for a steady supply of new genes during evolution.

*10) Pseudogenes or dead genes:* DNA sequences in these cases owe their origin to duplicated functional genes. They later become non-functional due to mutations. In some mammalian systems studied, they are located adjacent to each adult globin gene. These terms may be misnomers as their potential for giving rise to new genes cannot be disregarded.

*11*) *Nucleotypic DNA:* As against genotypic DNA, coding mainly for structural proteins, nucleotypic DNA controls certain biophysical parameters, such as, nuclear, cell and chromosome volume and duration of mitosis and meiosis. The increase in nuclear and cell size leads to an enlargement of the membrane, providing increased number of pores required in the transport of gene products. They undergo amplification and dispersion and are involved in the control of nuclear DNA processing, pairing, recombination and promotion of mutations. The role of highly amplified non-specific DNA, as a "padding" to keep chromatin in the folded state has also been assumed. The effect of nucleotypic DNA on biophysical operations may confer on it a selective value.

The properties of the different types of DNA, as enumerated above, obviously overlap. Almost all the non-unique sequences, not involved in coding for structural proteins, have the common attributes of *amplification, dispersion* and *mobility*. Mobility has been demonstrated in transposons and claimed for ignorant DNA and intervening sequences have been considered homologous to insertion elements. It is possible that this property is shared by all the DNA types, though it may remain demonstrated in some and undemonstrated in others. On the basis of these characteristics, the term *Dynamic DNA* was proposed embracing all the epithets applied so far. It would also include all the non-specific DNA sequences (Fig. VI: 1.2).

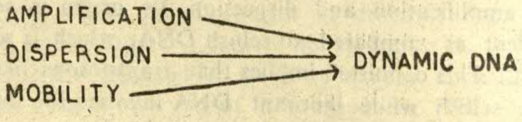

**Fig. VI: 1.2. Properties common to different types of non-unique DNA sequences not coding for structural proteins**

*Dynamic DNA*, endowed with the properties of amplification, dispersion and mobility, may even be involved in the control of unique sequences through moderate and minor repeat insertions. Its dynamic nature enables it to influence some of the vital functions of the cell, such as, regulation of mitotic and meiotic cycles, pairing, segregation, mutability, size of cell, nucleus and chromosomes, messenger processing and reshuffling and even the maintenance of the genetic skeleton. Its presence in multiple copies provides ample genetic stock for the generation of variability. It is no doubt a fact that evolution is not anticipatory. But absolute non-functional redundancy cannot be expected to survive under the rigours of natural selection, whether at phenotypic or cellular levels. The attribute of mobility and dispersion, enabling the DNA sequences to exist at multiple sites, is an evolutionary strategy to withstand elimination.

*Dynamic DNA* itself does not code for structural proteins, but the vital genes in which it is involved, are subject to stresses of physiological conditions. The flexibility of such DNA afforded by its amplified structure and mobility, is ideally suited to control genes requiring physiological adaptability. Though they may have possibly originated from unique sequences during evolution, yet the dynamic sequences are essential for the maintenance, functioning and evolutionary potential of the genetic apparatus. The repeated and intervening sequences and the transposons are manifestations of the three principal properties of Dynamic DNA, of amplification, dispersion and mobility respectively. The characteristics stressed in the other epithets, are all included within the definitions of Dynamic DNA (Sharma AK 1980).

## III. SOME ASPECTS AND PROSPECTS

### I. Genetic engineering and recombinant DNA

The scope of genetic engineering in obtaining improved races of domesticated plants and animals was understood long before the laws of heredity were officially formulated. The classical breeding practices, followed by man since the advent of civilization, in improving grain quality or yield in crops and in combining desired qualities in cattle or household pets fall within this topic.

However, the controversial aspect of genetic engineering came to the forefront with the initiation of Recombinant DNA research. Following public outcry, augmented through newspaper publicity, a moratorium was held on this type of work in the Asilomar Conference. Later, researches were permitted, within guidelines prescribed by the governmental committees, successively in the United States of America and the United Kingdom. Other developed countries followed suit. At the present date, researches are being carried out in different countries within the containments specified. The initial confusion having been cleared, it would be worthwhile to make a dispassionate survey of this rather touchy issue.

### *Recombinant DNA technology*

The utilisation of Recombinant technology entails the (i) isolation of a particular gene either by fragmentation of total genome or by transcribing meticulously purified species of mRNA through reverse transcriptase; (ii) availability of appropriate host; (iii) tailing of gene or DNA fragment which can reassociate with DNA fragments (vector) irrespective of origin; (iv) sealing of DNA fragments; (v) replication and maintenance of recombinant DNA molecules; (vi) introduction of recombinant DNA molecules into the host; (vii) selection of cells containing recombinant DNA molecules, and finally (viii) expression of the recombinant DNA within the host cell.

The phenomenon of bacterial transformation showed that genetic transformation occurred through the insertion of a single strand segment of input DNA into the resident DNA by displacing its homologous segment.

Bacterial transformation involves the direct uptake by a recipient strain of DNA isolated from a donor strain and its incorporation into the recipient's chromosome. Transduction is the transfer of bacterial DNA from donor to recipient mediated by a virus (bacteriophage). The bacterial DNA is packaged into a virus particle and is transferred to a new host cell on its infection by the virus. There are two main types of transduction. In one, generalised transduction, any part of the DNA of a donor cell may be transferred in this way. This is characteristic of viruses such as a coliphage PI. In others, for example the $\lambda$ phage of *E. coli*, the virus is able both to integrate into, and be excised from, the bacterial host chromosome. Once excised it multiplies vegetatively to give virus particles causing lysis of the host bacterium. When excision is aberrant, some of the host chromosome DNA is excised and packaged into vegetative particles of the virus and may subsequently be transferred to a recipient cell which is infected by the virus. Conjugation involves contact between a donor and recipient following which the whole or part of the chromosomal DNA of the donor may be transferred to the recipient strain. Such a process will also result in high frequency transfer of plasmids. These circular molecules of DNA occur naturally in many strains of bacteria. On an average they are approximately 1/100th the size of the bacterial chromosome. They may replicate autonomously to produce many copies per cell. Plasmid DNA may integrate into and be excised from the bacterial chromosome and aberrant excision may occur to yield plasmids carrying chromosomal genes. When transferred during conjugation, such plasmids carry the attached chromosomal DNA with them into recipient cells, which may be integrated into the host chromosome by recombination (see also earlier chapter).

The Pl R plasmids were found to have the ability to transfer themselves by conjugation between virtually any gram negative strains of bacteria and, therefore, were capable of promoting DNA transfer by strains not previously having this ability.

*In vivo* rearrangements of bacterial genes may be brought about by DNA elements termed *transposons* which can be transposed from one site to another within a bacterial cell, between and within chromosomes, plasmids or bacteriophage genomes. Their integration into new sites does not require nucleotide base sequence homology between the interacting DNA molecules. All such transposons have a repeated base sequence flanking antibiotic genes: these may be either direct or *inverted repeats*. Transposons when introduced into a bacterial strain can insert in any part of its DNA and thus cause mutation if inserted within the nucleotide sequence of a gene. The mutant strain will at the same time be 'tagged' with the antibiotic marker, and the location of the site of insertion can therefore be established by gene linkage analysis.

Knowledge of the above processes has led to techniques involving the *in vitro* construction of hybrid (or recombinant) DNA molecules which possess the ability to replicate autonomously when introduced into bacterial cells. Such molecules may consist of a foreign DNA fragment covalently linked to the DNA of either a bacterial plasmid or a bacteriophage vector.

The DNA fragment so linked may either be derived from another organism by fragmentation of its genome, or be synthesised *in vitro* either chemically or by the action of the reverse transcriptase enzyme on a messenger RNA molecule to yield copy or cDNA.

Such hybrid molecules may be introduced into bacterial cells, and transformed cells are selected by the action of auxotrophic drug or phage resistance genes carried as part of the molecule. Following their replication in transformed cells, many copies of these hybrid molecules can be isolated using standard plasmid or phage isolation procedures. The foreign DNA fragment of the molecule can then be separated from the plasmid or phage vector DNA. Should this fragment be that of a synthesised gene, cDNA, or a genomic fragment including a specific gene, this method may be used to clone specific genes away from their normal genetic background in such quantities as to make it possible to analyse them both structurally and functionally (see Fig. VI: 1.3).

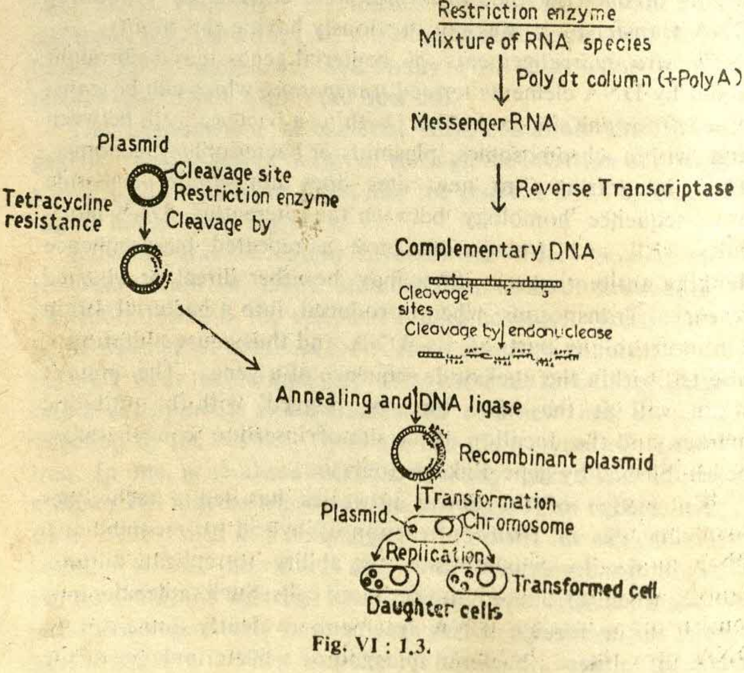

Fig. VI : 1.3.

The ability to clone foreign genes in bacteria has been made possible by combining a variety of different techniques. These include (a) the isolation and genetic modification of plasmid and bacteriophage vectors, (b) the 'cutting' of the *vector DNA* and the 'stitching in' of the foreign gene(s), (c) the infection of bacterial cells by the *hybrid DNA* molecules, and (d) the selection of those cells infected with the hybrid molecules carrying the foreign gene(s) which may or may not be expressed.

The successful development of techniques which permit the cloning and expression of foreign genes in bacteria has rested on the discovery of three new types of enzyme. Of them, *restriction endonucleases* and *ligases*, are involved in the construction of hybrid DNA molecules and their insertion into plasmid or phage vectors, and the other with the *in vitro* conversion of mRNA into cDNA molecules.

Attempts were made to induce such transformations in cells of higher organisms, using viruses and episomes as carriers of DNA. Human *gal*$^-$ cells were transformed to *gal*$^+$ with the help of lambda *gal*$^+$ transducing phage. However, these observations remained at the experimental phase till the identification of restriction enzymes.

Restriction endonucleases also play a part in the nucleotide sequencing of genes. Once a DNA fragment containing a known gene has been chosen and is available in large quantities, restriction endonucleases can be used to cleave the cloned gene fragment into smaller fragments amenable to *nucleotide sequencing*. Knowledge of the number and size of the fragments generated by different combinations of restriction enzymes permits the construction of a restriction endonuclease map which indicates the sequence of particular fragments making up the gene. That each such small fragment constitutes a part of the gene can be checked by its molecular hybridisation to a radioactive mRNA probe. The information derived from the restriction enzyme map, together with the nucleotide sequencing data of each small fragment, permits the nucleotide sequencing of whole genes. This type of information first showed that eukaryotic genes contain non-coding sequences: the cDNA derived from the mRNA transcript of the gene lacked these sequences.

There are two main types of restriction enzymes: those that cleave both strands of the DNA molecules at the same time, producing 'flush' ends, and those that cause staggered breaks thus resulting in fragments with single-stranded tails. The latter is generally used both to cleave the circular vector molecule to make it linear, and to produce DNA fragments for insertion into the vector. If the same restriction enzyme is used for both functions, the single-stranded tails will permit complementary base pairing and lead to the annealing of the two molecules. A *ligase* enzyme is then added to complete their union. Alternatively, covalent linkage of a DNA fragment and the vector molecule (made linear by restriction at a single site) can be brought about by synthesising oligo (dG) sequences at the 3′ terminal of the fragment by the action of the enzyme polynucleotide transferase, and oligo (dC) sequences at the 3′ terminal of the opened vector molecule, using the same enzyme. Recombinant molecules are formed by annealing the two types of DNA molecules which have complementary single stranded projections. The necessary exonucleolytic and ligative reactions are then carried out by the appropriate enzymes to yield the covalently linked hybrid DNA molecule.

The restriction enzymes, isolated from different bacteria, were seen to be capable of specific recognition of palindromic sequences and of cleaving DNA into specific fragments with small single-stranded ends. These ends could then be annealed with similar fragmented DNA molecules to give different genic combinations. Several such enzymes were identified e.g. Hind II from *Haemophilus influenzae* and Eco RI from *E. coli* (R). In case of a double-stranded DNA molecule without a restriction site, a synthetic ten deoxyribonucleotide double-stranded palindromic sequence with more than one target for restriction nuclease can be joined to both its sides by $T_4$ polynucleotide ligase. The resultant structure can be cleaved by endonuclease to give cohesive terminal ends, which in turn, are annealed to the vector DNA.

The other important enzyme which has contributed to clone genes in bacteria is reverse transcriptase. This enzyme acts *in vitro* to convert mRNA to a ssDNA copy, the reverse of that which normally occurs *in vivo*. The ssRNA can then be converted to a double stranded DNA molecule by the action of a polymerase enzyme. Reverse transcriptase is isolated from cells infected with RNA viruses: the virus uses the enzyme to convert its RNA into DNA after infection of the cell. Since the m-RNA transcript of the gene is the substrate, only the DNA coding sequences are included in the synthetic gene; and the intron non-coding sequences are omitted.

A specific gene for inclusion in a plasmid or phage vector may also be synthesised chemically from oligonucleotides corresponding to the genetic codes and sequences of the amino acids composing the polypeptide product of a genomic gene.

The other breakthrough which accelerated Recombinant DNA research was the identification of *versatile cloning vectors.* Small plasmids, each with a replicating region and a specific antibiotic resistance as marker, were isolated, like pSC101 and Col El and the more recent pMB9 and pCR1. DNA from lambda phage was tailored for inserting and cloning foreign DNA.

By removing specific DNA segments with restriction endonucleases, hooking them with a suitable vector and final transferance into the host DNA, many successful transfers of prokaryotic DNA into *E. coli* have been carried out. Even rat insulin gene has been cloned in *E. coli* (Ullrich A *et al.* 1977 *Science* **196**: 1313). In some cases, the inserted eukaryotic DNA sequence has been expressed in the prokaryotic cell, as for example, yeast genes controlling histidine and leucine biosynthesis in *E. coli* cells (see reports of Symposium on Molecular Cloning of Recombinant DNA, Miami, Florida 1977).

Although it has proved relatively simple to integrate eukaryotic genes or their coding equivalents (cDNA and synthetic gene) into plasmids and transfer them into *Escherichia coli*, their transcription and translation to produce a normal gene product have proved more difficult, since the protein-synthesising machinery of the two is different. Nevertheless, two main approaches have been used and proved to be successful in 'switching on' both the transcription and translation of eukaryotic genes in *E. coli.* In both cases the genetic information surrounding the eukaryotic gene sequences has been chosen so as to make the eukaryotic gene sequence subject to the regulatory commands of bacterial gene coding sequences. In the first approach the cloned eukaryotic gene is spliced into a bacterial gene (also integrated into the vector DNA molecule) so that the bacterial gene initiation codon is followed directly by the codon for the first amino acid of the eukaryotic protein. The human leucocyte interferon has been produced by *E. coli* following the fusion of the cDNA to the bacterial tryptophane operon already inserted in the plasmid vector. The second and more commonly adopted approach is to insert the eukaryotic gene into an internal position in a bacterial gene already carried by the vector so that the sequences of the inserted gene are in phase with the bacterial coding sequences, thus resulting in the production of a bacterial eukaryotic hybrid protein, which can then be released from the hybrid protein *in vitro*. This method has been used successfully in the production by *Escherichia coli* of many mammalian proteins including rat proinsulin, β-endorphin and rat growth hormone. It has the advantage that the eukaryotic protein when combined with a hybrid protein may be protected from degradation by bacterial enzymes; the particular bacterial protein can then be selected so as to contain a N-terminal sequence permitting the hybrid to be transmitted to the cell surface thereby facilitating its recovery and its detection by immunological methods employing fluorescent or radioactively labelled antibodies. Each restriction enzyme recognises a specific nucleotide sequence wherever it occurs in a DNA molecule and cleaves it at or near each recognition site to produce fragments. The number of fragments produced by a particular endonuclease, therefore, depends on the number of recognition sites in the original DNA molecule. The size (number of nucleotide bases) and the nature (base sequence) of the sites recognised by different endonucleases vary; most have a recognition site 4-6 nucleotide base pairs long.

The transfer and functioning of genes in combination with alien DNA in alien cytoplasm led to both jubilation and misgivings about the future of this type of work.

These novel genetic combinations can be undoubtedly of benefit in understanding the structure and function of the eukaryotic chromosome, if pre-arranged segments can be removed and cloned and studied individually. Another possibility is the transfer of a gene controlling a necessary protein, like insulin or hormones to a bacterium, where its rapid replication with the host cell would lead to the production of large quantities of the protein concerned. The same principle can be applied to the manufacture of vaccines.

Nitrogen fixation in nature is restricted to a few bacteria belonging to the genera like *Azotobacter* and *Klebsiella*, which may exist in association with higher plants. All these bacteria possess genes for nitrogen fixation (*nif*). This capacity may be increased by multiplying the number of *nif* genes in the bacterial cell through hooking *nif* genes on an amplifiable plastid and introducing it into the bacterium. A beginning has been made by inserting *nif* genes from *Klebsiella pneumoniae* into *E. coli* through a plasmid RP 41. Another probability, rather remote at present, is of direct transfer of *nif* genes to plant protoplasts.

An important application of Recombinant DNA research is in the therapy of gene-controlled diseases. As mentioned in chapter IV: 5, of the nearly 1500 or more genetic disorders identified, quite an appreciable number exhibits single gene control, leading to lowered or altered protein synthesis for which the particular gene is responsible. The addition of chromosomal material is possible *in vitro* in somatic cell hybridisation studies. The possibility of the use of Recombinant DNA has been shown by transferring the gene for thymidine kinase to human cells deficient in the enzyme with the help of herpes simplex viral DNA. The limitation of the technique is the difficulty in returning the transformed cells to the human body under *in vivo* conditions (Bacchetti S and Graham FL 1977 *PNAS USA* **74**: 1490).

The less pleasant consequences of this type of research, which have so powerfully agitated public opinion, are two fold. The first apprehension is that such experiments may lead to the transfer of genes harmful to human health to *E. coli* or other host DNA. Any accidental release of such a form in the environment will lead to problems of disease management and biohazards. This fear was particularly aggravated in view of the "shotgun" experiments, in which the DNA is fragmented nonspecifically. The guidelines laid down to prevent this type of risk specify two types of containments: physical and biological. The former involves degrees of sterilisation and isolation and the latter the use of different combinations of weakened hosts and vectors. Certain combinations are forbidden. Advocates of this type of research are of the opinion that the hazards are imaginary and that recombinations occurring in nature are more varied than can ever be produced in a laboratory.

The other danger attributed to genetic engineering is the possibility of producing so-called "monsters" by manipulating genes at the zygotic or pre-zygotic level. It is still at the stage of conjecture since as yet, it has not been possible to bring a human zygote to the full term baby under cultural conditions. In addition, only a very small fraction of the 20,000 to 40,000 pairs of human genes have been mapped (see list in appendix). Therefore tampering with the genetic constitution of a zygote is still beyond the reach of the geneticist, and likely to remain so, for a considerable period of time (Table VI: 1.1).

**TABLE VI: 1.1**

**Some potential applications of recombinant DNA technology (from Bevan EA 1981 In:** ***Impact of the development of science and technology on environment,*** **Indian Science Congress Association Calcutta)**

| *A. Production of therapeutic and preventive drugs* | *Consequences* | *Possible examples* |
|---|---|---|
| Hormones | Increased yield and cheaper production | Antibiotics against Pseudomonas, Serratia and Proteus |
| Antibiotics | Increased animal productivity by their use | Pituitary growth hormone |
| Vaccines | Synthesis of new and more effective drugs | Factor VIII<br>Urokinase |
| | Safer vaccines—Pathogen not handled during production | Foot and mouth vaccine, Swine fever vaccine, etc. |
| | Free from toxic and pyrogenic components | |
| *B. Enhancement of enzyme production by bacteria concerned with:* | | |
| 1. Biodegradation of natural wastes | Products converted into animal feeding stuff | By-products of oil industry<br>N-alkanes and methane |
| a. Domestic refuse and sewage | Source of energy | Animal feed |

*Contd. (Table VI: 1.1)*

| *A. Production of therapeutic and preventive drugs* | *Consequences* | *Possible examples* |
|---|---|---|
| b. Organic by-products of industry | Cost of disposal reduced<br>No pollution | |
| c. Agricultural wastes | | |
| 2. Biodegradation of synthetic products, e.g. pesticides and herbicides | Pollution reduced e.g. Dieldrin cost reduced | Enzyme modification of Arthrobacter, Bacillus, Nocordia and Pseudomonas spp. |
| 3. Trapping of toxic materials present in industrial wastes | Recovery of costly materials<br>Pollution reduced | Cu and Ag recovered |
| 4. Oxidation of hydrocarbons in industrial processes | Conservation of energy: lower temperature and pressure needed than in conventional methods; lower costs<br>Pollution reduced | Modification of enzyme production of Methylococcus capsulatus which oxidises: Ethylene—Ethylene Oxide<br>Propylene—Propylene Oxide<br>Benzene—Phenol<br>Toluene—Cresol, etc. |
| 5. Production of single cell protein (Bacteria =50-80%) | Modification for enzymes concerned with, for example, ammonia assimilation | |
| 6. Analysis of restriction endonuclease digests of DNA derived from: | | |
| a. Different pathogenic virus strains | Strains distinguished giving evidence for their origin | Influenza virus |
| b. Human foetal cells derived from amniotic fluid | Diseases in foetus diagnosed (amniocentesis) | |

As the case stands now, the benefits to be derived from Recombinant DNA research far outweigh the mishaps anticipated. Such work should therefore be permitted, under adequate biological and physical containments.

## II. Banding pattern analysis

Banding patterns, which serve to identify individual chromosome segments, are being continually devised. However their application is usually limited to specific groups or even taxa. The most numerous ones have been devised on the mammals. No single technique has a universal application.

### *In the study of evolution*

The importance of banding patterns in studying evolution in both animal and plant groups is obvious, since they present finer and more precise parameters for the comparison of karyotypes. Homologies can be demonstrated on the basis of specific patterns as also in the presence of particular criteria, like the satellite DNA. The repeated sequence DNA contents can be estimated by DNA renaturation kinetics using hydroxyapatite chromatography for separation of renatured from denatured DNA. A comparative analysis of such fractions in related genomes can show the changes involved in their evolution, as in several cereals. Attention is being drawn to the so-called satellite DNAs, since of the different types of repetitive sequences, these can be readily purified for study. Such DNA is shown to be located in constitutive heterochromatin, through *in situ* hybridisation techniques. Their analysis has aided in tracing the evolution of the higher groups of plants and animals, notable examples being the B genome in wheat and higher primates.

Variations in the location and size of Q-stained regions and C-bands between homologous chromosomes, without any associated phenotypic effects, have been observed in primates. The gorilla is most striking, having polymorphisms of at least half the chromosomes. Such polymorphisms also exist, with regard to the fluorescent intensity of variant Q-bands in human newborns. The bands can be effectively employed as markers in pedigree analysis, for population studies and for gene localisations. The reverse banding technique has emerged as a powerful tool for gene localisation.

*In the study of diseases*

There has been a veritable explosion of reports on the application of the banding schedules in the study of clinical diseases. The greater precision in the identification of the chromosome segments shows promise in correlating many new clinical syndromes with segmental changes, including the role of chromosomal alterations in maldevelopment, mental retardation, abortions, behavioural disorders and perhaps also in cancer. These patterns are being progressively employed in new experimental approaches, using heteroploid and somatic hybrid cell lines.

*Somatic cell hybridisation*

The most impressive progress has been made in mapping chromosomes in widely used animal species, like mouse. The banding techniques have also aided tremendously in assigning genes to human chromosomes. Using rodent-human somatic cell hybrids from which human chromosomes tend to be lost, the construction of genetic maps in mouse and man is the prelude to extensive understanding of linkage relationships in other eukaryotic species.

The karyotype analysis of interspecific cell hybrids is, however, often complicated by the large number of chromosomes in the heterokaryons. In the first stage, staining techniques are used to broadly differentiate the parental origin of individual chromosomes or determining rapidly the number and morphology of the chromosomes of interest. These chromosomes are then characterised precisely by conventional banding techniques.

The application of enzyme electrophoresis and differential chromosome staining methods in genetic analysis of human-rodent hybrid cell lines has led to the assignment of different enzyme loci to the human chromosomes. The accompanying table gives the genes assigned to the respective chromosomes in the human karyotype (Table VI : 1.2).

## III. Genetics in the study of man

Some further applications of genetics in the study of man are:

*Linkage studies*: From the map provided by serological polymorphism and electrophoretic analysis of protein variants, linkage relations would be able to indicate the likelihood of an individual being affected by unifactorial diseases with manifestation late in life.

*Detection of heredity through correlation coefficient between two variable characters and twin study*: In general, there is no doubt that ordinary mental qualities are controlled by normal genetic means, being determined jointly by heredity and environment as are physical characters. However, the nature and the pattern of inheritance of these characters can be studied successfully.

*Eugenics*: Very rare recessive conditions are extremely widespread and therefore impossible to be rid of in a population. Heterozygous defects can be grouped into two classes: those which reduce significantly the number of children born to affected individuals and those which do not. The first of these, as for example, Darrier's disease, can never be controlled in a population by eugenic measures since it is caused by mutation of the gene involved. In the second category, eugenic measures may have certain influence. Multifactorial conditions, on the other hand, provide much better opportunities for eugenics. Mental abnormality and deficiency belong mostly to this category and can be substantially reduced through a programme of sterilisation. Positive eugenics, or the dissemination of advantageous genes, has also been successfully employed in plants and animals to segregate and eliminate the disadvantageous recessives by close inbreeding. The application of this process in man may obviously be made at a distant future.

*Possible damage to the genetic make-up of man* has received progressive attention since the turn of the century. The rate of recurrent mutations is increasing with the advancing technology, from the environmental pollutants released in the food, water and air. New surgical techniques and treatment of congenital metabolic disorders have contributed to relaxation of natural selection, challenging the mutation-selection balance which has hitherto controlled biological adaptation.

*Clinical genetics*

The practice of medicine seeks the answer to three questions. What is wrong? The answer is diagnosis, aided by applied cell biology in the form of the study of cultured skin fibroblast and banding patterns of chromosomes, of pinpointing the region responsible, also the methods for linkage studies and of identifying the gene responsible in inborn errors of metabolism.

What is going to happen? The answer is prognosis, which is the basis of genetic counselling, helped by prenatal diagnosis made possible by the culture and study of amniotic cells for genetic aberrations.

Why did it happen? The answer involves environmental factors, internal, external and social.

What can be done about it? The answer is treatment—palliative and/or curative. Closely allied is prevention. Both have been aided by newer knowledge of the basic defect. Programmes for prevention of recessive diseases by identification of carriers have been designed. The prospects of gene therapy and cure of cancer through somatic cell hybridisation are becoming brighter.

*Genetic diagnosis of molecular diseases and others*

Precision has been attained in some biochemical abnormalities and chromosomal aberrations. However multifactorial diseases should be looked at in relation to the normal distribution of the respective traits (see chapter IV:5).

*Genetic counselling* is rapidly developing due to the reduction in infant mortality caused by environmentally controlled diseases. The diseases with genetic causes, (nearly 2000 are at present known), are coming into focus also due to their increased survival rate. A sense of responsibility for healthy children is being instilled in parents with the concept of planning for a small family. There is a spread of relevant knowledge amongst the public and also a consciousness that with effective new treatments, such disorders will show a gradual increase. At the present state, genetic counselling seeks to inform parents of an affected child of risk to future offspring, medical practitioners of the special risks to an yet unborn child to facilitate diagnosis and treatment and to prevent, in the long run, an increase in the birth frequency of individuals with such disorders.

Advent of genetic studies following amniocentesis, through which a diagnosis may be made of several genetical abnormalities in the foetus at the 14th to 16th week of pregnancy, has greatly aided genetic counselling, particularly in cases of high risk. Another very desirable improvement is the progressive detection of clinically normal heterozygotes, particularly for dominant conditions of late onset, in addition to X-linked and autosomal recessives. The future control of genetically determined disease is likely to be mostly through prenatal screening by amniocentesis for chromosomal anomalies; genetic counselling supplemented by prenatal diagnosis for conditions due to mutant genes of large effect and detection of those genetically at risk and special environmental prophylaxis for conditions determined multifactorially.

Mammalian gene mapping techniques can contribute significantly to prenatal diagnosis and to human molecular genetics. Restriction fragment mapping can be used to place polymorphic genetic markers at random sites within the genome. These sites can be used to assign genes responsible for disease conditions to a chromosomal region. Somatic cell genetic techniques can then be applied to saturate that region with additional restriction fragment markers, some of which will be closely linked to the disease gene. Closely linked restriction fragment markers, adjacent to pairs of markers, can act as predictors for the transmission of defective genes to offspring. A series of tightly linked flanking restriction markers may also lead to the ultimate isolation and cloning of the disease gene itself (see Ruddle FH 1981 *Nature* **294**: 115).

## Prospects of gene therapy

Recombinant DNA technology has made it possible to isolate, study and identify genes coding for particular proteins in the laboratory and in consequence to identify abnormalities caused by mutations. Identification of a serious gene defect in an individual opens up two possibilities: (a) to prevent the birth of the conceptus if identified *in utero* within the first few weeks, and (b) to replace the defective gene by a functional one. The former is an acceptable and effective practice. The latter, though not yet possible, may become possible soon (see Williamson B

1982 *Nature* **298**: 416).

In a few diseases with single gene defect, like sickle cell anaemia, thalassaemia, growth hormone deficiency, some collagen disorders and Lesch-Nyhan syndrome, altered DNA sequences have been cloned into bacterial plasmids. These can be used to identify whether an individual is homozygous or carrier of the defective gene and are, therefore, disease specific cloned probes. Gradually other single gene defects are being added to the list. Antenatal diagnosis of single gene and chromosomal defects in the foetus gives the option of medical termination of pregnancy, which is the usual procedure.

Gene therapy, on the other hand, must involve the insertion of a functional human gene into the relevant tissue, where it can respond to the normal functioning of the body. The successive stages required are: identification of the defective gene, isolation of a normal counterpart and development of a method to put the normal gene into a cell where it can function. It may even be required to insert the gene into the correct site on the host cell chromosome or even to delete the defective gene. The latter part of the programme is not yet feasible but may become so within the foreseeable future. There will be two aspects: (1) treatment of a serious disease so that only the patient is affected, and (2) treatment of an early embryo so that its inheritance is altered. Before adopting the procedures, the ethical aspects should also be taken into account.

### *Ultrastructural studies*

Ultrastructural cytogenetics is a developing branch of cell biology. Standard electron microscopy complemented by cytochemical analysis is employed in obtaining information on chromosome relationship and behaviour. The possibility of constructing pachytene idiograms is being explored based on the length and other characteristics of synaptonemal complexes from EM tridimensional reconstruction of nuclei. Whole mount human chromosomes facilitate the study of interchromatid and interchromosomal fibres.

Three areas may be recognised in which scanning and computing machinery may assist in the work of a chromosome laboratory. They include screening to eliminate all apparently normal subjects from the population being examined for karyotypic abnormality; counting for a statistical assessment of mitosis, chromosomal changes, polyploid cells and hot spots; and accurate measurement of length, area or integrated optical density of the different regions of the chromosomes.

Image analysing computers using Video imaging and digital logic techniques are being further used in biology. Recent applications include the direct presentation of chromosome banding patterns, the analysis of nuclear chromatin distribution and the use of fluorescence techniques.

## Conclusions

The study of chromosomes has advanced far from the initial stages of chromosome counts after a laborious processing. Since the chromosomes are the bearers of genes, the ultimate units of heredity, a study of gene expression necessarily involves the handling and observation of the chromosomes.

Chromosome science has developed apace due to advancements in technology and microscopy. It is, of necessity, a synthetic one, with contributions from cytology, genetics, biophysics and biochemistry, and more recently medicine and computer science, amongst others. Its ramifications are spread over a wide range of interrelated topics as well. JBS Haldane, in 1954, predicted "the chemical structure of chromosomes is as detailed as that of a book or picture and key to a knowledge of that structure is the science of genetics". His prediction has been fulfilled beyond expectation.

The contributions of cytogenetics and breeding to human welfare are rooted in the knowledge of their chromosomes. An understanding of the structural and behavioural patterns of chromosomes has eliminated most of the pitfalls in the tedious process of breeding, leading to revolutions in food, fodder and fuel production. Karyotype analysis has been widely employed in cytotaxonomy—in describing the phylogeny, evolution and interrelationships of taxa in both animal and plant kingdoms. The study of chromosomal polymorphisms has led to an understanding of the role of natural selection in speciation. The effects of different physical and chemical agents on chromosomes are utilised in various ways. Since even distilled water appears to cause alterations in chromosome behaviour, the dangers of careless technological progress and of environmental pollution, both external and internal, on the genetic system, are emphasised. The differential response of the same drug on different races is the basis for the formation of the science of pharmacogenetics. The basic nature of the chromosomes, as observed through biophysical and biochemical methods, through electron microscopy and computerisation of data, may ultimately result in the reconciliation of molecular and microscopical observations.

The principal problem which faces the genetic counsellor and the genetic engineer is how genetic knowledge may be employed in improving the lot of mankind. Dizzy vistas have been opened up of cure of inherited defects or even addition of desirable genes. They are still not very close due to many technical limitations at present. Several practical difficulties are faced in the replacement of a defective gene. The chromosome map does not pinpoint the actual location of a particular DNA segment in most eukaryotes. The substitution of a mutant gene by normal allele in somatic cells, if made possible, may alleviate the suffering of the individual but cannot be transferred to the next generation. The construction of a specific gene *to order* from nucleotide units as yet has not been fully blueprinted. The final step is that of injecting the synthesised gene into the cells of the victim carrying the defective gene. This step may possibly be accomplished through virus or plasmid mediated technology.

Cell fusion may be applied for this purpose. By fusing cells of two species it may be possible to transfer genes to the deficient line to have a normal line. Hybridisation between two malignant cell lines may similarly result in the formation of a non-malignant one. Parasexual hybridisation may give rise to otherwise infertile interspecific crosses.

The dynamism exhibited by the chromosome in its structure and behaviour presents an optimistic future for possible artificial control of chromosomal behaviour and subsequently, its evolution.

The tendency of chromosomes to undergo changes may be employed advantageously in the origin of life forms useful to mankind. It may become possible to manipulate such chromosomal alterations to the extent that a particular modification is required. From this viewpoint, the culture of single chromosomes assumes importance. Single chromosomes have been obtained *in vitro* but survival in culture and gene expression are yet to be achieved. With the elaboration of this technique it may become possible to determine the exact location and regulatory system of individual genes and manipulate them with predictable accuracy.

**Appendix : TABLE VI: 1.2. Human genes mapped up to 1983**

Catalog of mapped genetic markers and linked genes[1] (in Human Gene Mapping 7 Los Angeles Conference, ed Sparkes RS *et al Cytogenet Cell Genet* 37: 1 1984)

| *Marker symbol*[2] | *Previous symbol*[3] | *Marker Name* | *E.C. No.* | *Category*[4] | *Chromosomal region or linkage group*[5] |
|---|---|---|---|---|---|
| *1* | *2* | *3* | *4* | *5* | *6* |
| A12M1 | | adenovirus-12 chromosome modification site IC | | P | 1q42-q43 |
| A12M2 | | Do site 1A | | P | 1p36 |
| A12M3 | | Do site 1B | | P | 1q21 |
| A12M4 | | Do site 17 | | P | 17q21-q22 |
| AABT | | beta-amino acid transport | | P | 21 |
| ABL | | Abelson murine leukemia viral (v-abl) oncogene homolog | | C | 9q34 |
| ABO | | ABO blood group | | C | 9q34 |
| ACAD | | acyl CoA dehydrogenase, multiple; glutaric aciduria II | 1.3.99.3 | P | X |
| AC01 | | aconitase 1, soluble | 4.2.1.3 | C | 9p22-p13 |
| AC02 | | aconitase 2, mitochondrial | 4.2.1.3 | C | 22q11.2-q13.31 |
| ACP1 | | acid phosphatase 1, cytoplasmic | 3.1.3.2 | C | 2p25 or 2p23 |
| ACP2 | | acid phosphatase 2, lysosomal | 3.1.3.2 | C | 11p12-cen |

*Contd.* (*Appendix : Table VI: 1.2*)

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| ACT1, ACT2 | | actin like sequence | | P | Xp11-q12, Y |
| ACT3 | | actin like sequence | | P | 2p23-qter |
| ACT4 | | actin like sequence | | P | 3pter-q21 |
| ACTA | | actin, alpha, skeletal muscle | | C | 1p21-qter |
| ACTC | | actin, alpha, cardiac muscle | | P | 15q11-qter |
| ACY1 | | aminoacylase 1 | 3.5.1.14 | C | 3p21 |
| ADA | | adenosine deaminase | 3.5.4.4 | C | 20q13.2-qter |
| ADCP1 | | adenosine deaminase complexing protein 1 | | I | 6 |
| ADCP2 | | Do protein 2 | | C | 2 |
| ADH | | alcohol dehydrogenase, class I | 1.1.1.1 | P | 4 |
| ADK | | adenosine kinase | 2.7.1.20 | C | 10cen-q24 |
| ADRBR | | beta-adrenergic receptor, surface | | P | 5 |
| ADS | | albinism-deafness syndrome | | C | X |
| AF8T | | AF8 temperature sensitivity complementing | | P | 3 |
| AFP | | alpha-fetoprotein | | P | 4q11-q22 |
| AHC | | adrenal hypoplasia, congenital | | P | X |
| AHCY | | S-adenosylhomocysteine hydrolase | 3.3.1.1 | C | 20cen-q13.1 |
| AHH | | aryl hydrocarbon hydroxylase | 1.14.14.1 | C | 2 |
| AIC | | Aicardi syndrome | | P | Xp22 |

| | | | | |
|---|---|---|---|---|
| AIH | amelogenesis imperfecta, hypomaturation or hypoplastic | | P | X |
| AK1, AK2, AK3 | adenylate kinase 1, 2, 3 | 2.7.4.3 | C | 9q34, 1p34, 9p24-p13 |
| ALAD | delta-aminolevulinate dehydratase | 4.2.1.24 | C | 9 |
| ALAS | delta-aminolevulinate synthetase | 2.3.1.37 | P | X |
| ALB | albumin | | C | 4q11-q22 |
| ALD | adrenoleukodystrophy | | C | X |
| AMY1 | alpha-amylase, salivary | 3.2.1.1 | C | 1p21 |
| AMY2 | alpha-amylase, pancreatic | 3.2.1.1 | C | 1p21 |
| AN2 | aniridia without Wilms tumor, genitourinary abnormalities, and mental retardation | | P | 11p13 |
| APOA1 | apolipoprotein A-I | | C | 11p11-q13 |
| APOA4 | apolipoprotein A-IV | | P | 11 |
| APOC3 | apolipoprotein C-III | | C | 11p11-q13 |
| APOE | apolipoprotein E | | C | 19 |
| APRT | adenine phosphoribosyltransferase | 2.4.2.7 | C | 16q12-q22 |
| ARSA, ARSB | arylsulfatase A and B | 3.1.6.1 | C | 22q13.31-qter and 5 |
| ASB | anemia, sideroblastic/hypochromic | | C | X |
| ASL | argininosuccinate lyase | 4.3.2.1 | P | 7p21-q22 |
| ASNS | asparagine synthetase | 6.3.5.4 | P | 7p11-q11 |
| ASS | argininosuccinate synthetase | 6.3.4.5 | C | 9q34-qter |
| ASSP2 | pseudogene 2 | | P | 6 |

Contd. (*Appendix: Table VI: 1.2*)

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| ASSP4 | | pseudogene 4 | | P | X |
| ASSP5 | | pseudogene 5 | | P | X |
| ASSP6 | | pseudogene 6 | | P | Y |
| AT3 | | antithrombin III | | C | 1q23 |
| ATN | | albinism, tyrosinase-negative | | | LG9 |
| ATPM | | ATPase, mitochondrial | | P | 10 |
| AVR | | antiviral state regulator | | P | 16 |
| AVVR | | antiviral state repressor regulator | | P | 5p |
| B2M | | beta-2-microglobulin | | C | 15q21-q22 |
| B2MR | | beta-2-microglobulin regulator | | C | 15q13-q15 |
| BA2R | | BALB/c 3T3 ts2 temperature sensitivity complementing | | P | Xq13-q27 |
| BCT1, BCT2 | | branched chain aminotransferase 1 and 2 | 2.6.1.26 | C | 12pter-q12 and 19 |
| BEV1 | | baboon M7 virus integration site | | C | 6 |
| BF | | properdin factor B | | C | 6p21.3 |
| BLVR | | biliverdin reductase | 1.3.1.24 | C | 7p14-cen |
| BMD | MDB | muscular dystrophy, Becker type | | C | X |
| BORJ | | Borjeson syndrome | | P | X |
| BR1A, B | ITG1, 2 | blastogenic response to synthetic polypeptide (T,G)-A—L | | P | 6 |

| | | | | | |
|---|---|---|---|---|---|
| BR2A, B | IHG1, 2 | blastogenic response to synthetic polypeptide (H,G)-A—L | | P | 6 |
| BR3A, B | | blastogenic response to synthetic polypeptide (phe,G)-A—L | | P | 6 |
| BR4A and BR4B | | blastogenic response to synthetic polypeptide GAT | | P | 6 |
| BR5A and 5B | | blastogenic response to synthetic polypeptide GLphe | | P | 6 |
| BVIX | | BALB virus induction, xenotropic | | P | 11 |
| C1HR | | C1AGOH temperature sensitivity complementing | | P | X |
| C2 and C3 | | complement component 2 and 3 | | C | 6p21.3 & 19pter-p132 |
| C3BR and DR | | complement component 3b and 3d receptor | | P | 6p21.3 |
| C4A | C4F | complement component 4A | | C | 6p21.3 |
| C4B | C4S | complement component 4B | | C | 6p21.3 |
| C6 and C7 | | complement component 6 and 7 | | | LG6 |
| C8 | | complement component 8 | | C | 1p |
| CA2 | | carbonic anydrase 2 | 4.2.1.1 | P | 8 |
| CA21H | CAH | congenital adrenal hyperplasia, (21-hydroxylase deficiency) | | C | 6p21.3 |
| CAE | | zonular pulverulent cataract (Fy linked) | | C | 1cen-q25 |
| CAT | | catalase | 1.11.1.6 | C | 11p13 |
| CBBM | | color blindness, blue monochromatic | | P | Xq |

Contd. (*Appendix: Table VI: 1.2*)

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| CBD and CBP | | color blindness, deutan and protan | | C | Xq27-qter |
| CBS | | cystathionine beta-synthetase | 4.2.1.22 | P | 21 |
| CCAT | | congenital cataract | | | LG7 |
| CCT | | congenital cataract, total | | C | X |
| CGA | | chorionic gonadotropin, alpha polypeptide | | P | 6q11-q21 |
| CGB | | Do, beta polypeptide | | C | 19 |
| CGD | | chronic granulomatous disease | | C | Xpter-p21 |
| CGH | | chorionic gonadotropin | | P | 18 |
| CHE1 | | cholinesterase (serum) 1 | 3.1.1.8 | | LG1 |
| CHR | | chromate resistance; sulfate transport | | P | 5q35 |
| CKBB | | creatine kinase, brain form | 2.7.3.2 | C | 14q32-qter |
| CLA | | cerebellar ataxia (autosomal recessive) | | | LG9 |
| CLA2 | | cerebellar ataxia 2 | | C | X |
| CLG | | collagenase, epidermolysis bullosa, recessive dystrophic | | P | 11 |
| CLPA | | cleft palate | | P | X |
| CLS | | Coffin-lowry syndrome | | P | Xpter-p12 |
| CMT1 and 2 | | Charcot-Marie-Tooth neuropathy 1 and 2 | | C | 1q and X |

| | | | | |
|---|---|---|---|---|
| COL1A1, COL1A2 | collagen, type I, alpha 1 and 2 | | C | 17q21-q22, 7q21-q22 |
| COL3A1 | collagen, type III, alpha 1 | | T | 7 |
| COLM | collagen, alpha 1-like | | P | 12 |
| CON1 and CON2 | concanavalin A reactive salivary protein 1 and 2 | | | LG4 |
| CP | ceruloplasmin | | | LG1 |
| CPA | carboxypeptidase A | 3.4.2.1 | P | 7 |
| CPSD | cathepsin D | 3.4.23.5 | P | 11pter-q12 |
| CRD | choroido-retinal degeneration | | C | X |
| CRP | C polysaccharide-reactive protein | | P | 1 |
| CS | citrate synthase | 4.1.3.7 | C | 12p11-qter |
| CSH1 and CSH2 | chorionic somatomammotropin hormone 1 and 2 | | C | 17q22-q24 |
| CSHP1 | chorionic somatomammotropin pseudogene 1 | | C | 17q22-q24 |
| CTH | cystathionase | 4.4.1.1 | P | 16 |
| CTRB | chymotrypsinogen B | 3.4.21.1 | P | 16 |
| D1S1 | DNA segment, single copy, probe H3 | | C | 1p36 |
| D1S2 | Do, probe L1.22 | | P | 1 |
| D121 | DNA segment, repetitive, probe HS3 | | C | 1q12 |

Contd. *(Appendix: Table VI: 1.2)*

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| D2S1 | | DNA segment, single copy, probe L2.30 | | P | 2 |
| D2S2 | | Do probe L10 | | P | 2 |
| D3S1 | | Do probe HS3 | | C | 3q12 |
| D3S2 | | Do probe pHF-12-32 | | P | 3q21-qter |
| D3S3 | | Do probe pMS1-37 | | P | 3p |
| D3S4 | | Do probe B67 | | P | 3 |
| D4S6 | | Do probe 4Cm3 | | P | 4 |
| D4S9 | | Do probe 4ER1 | | P | 4q |
| D4S10 | | Do probe G8 | | P | 4 |
| D5S1 | | Do probe L1.7 | | P | 5 |
| D5S2 | | Do probe pHF12-65 | | P | 5pter-q35 |
| D5S3 | | Do probe pHD1 | | P | 5 |
| D6S1 | | Do probe B120 | | P | 6q21-qter |
| D6S2 | | Do probe p2.2 | | P | 6q |
| D6S3 | | Do probe 12.56 | | P | 6 |
| D6S4 | | Do probe 4c11 | | P | 6p11-cen |
| D6S5 | | Do probe pAG-B6 | | P | 6p24-q12 |
| D6S7 | | Do probe p7H4 | | P | 6p |
| D6S8 | | Do probe p2C5 | | P | 6p |
| D6S9 | | Do probe p3C7 | | P | 6p |

| | | | |
|---|---|---|---|
| D6S10 | Do probe pCH6 | P | 6p |
| D7S1 | Do probe pA2H3 | P | 7 |
| D7S2 | Do probe p7-15 | P | 7 |
| D7S3 | Do probe p7-11 | P | 7 |
| D7S4 | Do probe p7-12 | P | 7 |
| D7S5 | Do probe p7-13 | P | 7 |
| D8Z1 | DNA segment, repetitive, probe LI.26 | P | 8 |
| D9S1 | DNA segment, single copy, probe p12-8 | P | 9 |
| D9S2 | Do probe 9ER1 | P | 9q |
| D9Z1 | DNA segment, repetitive, probe QP23 | P | 9qh |
| D9Z2 | Do probe pPD9 | P | 9p13-q13 |
| D10S1 | DNA segment, single copy, probe Dry5-1 | P | 10 |
| D10S2 | Do probe p3-10 | P | 10 |
| D11S1 | Do probe H11-17 | P | 11p11-q13 |
| D11S3 | DNA segment, single copy, probe H11-8 | P | 11p13-p12.08 |
| D11S4 | Do probe H11-19 | P | 11p11-q13 |
| D11S5 | Do probe H11-13 | P | 11p11-q13 |
| D11S6 | Do probe H11-12 | P | 11p13-p12.08 |

Contd. (*Appendix: Table VI: 1.2*)

| *1* | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| D11S7 | | Do probe H11-6 | | P | 11p11-q13 |
| D11S8 | | Do probe H11-9 | | P | 11p12-p13.08 |
| D11S9 | | Do probe H11-22 | | P | 11p12.08-p11 |
| D11S10 | | Do probe H11-39 | | P | 11p13-p12.08 |
| D11S11 | | Do probe H11-21 | | P | 11p12.08-p11 |
| D11S12 | | Do probe pADJ762 | | C | 11p |
| D11S13 | | Do probe not specified | | P | 11 |
| D11S14 | | Do probe H11-20 | | P | 11p12.08-p11 |
| D12S1 | | Do probe L.2.2 | | P | 12 |
| D12S2 | | Do probe p12-16 | | P | 12 |
| D12S5 | | DNA segment, single copy, probe p12-2.2 | | P | 12pter-p12.05 and q13-q15 |
| D13S1 | | Do probe p7F12 | | P | 13q12-q14 |
| D13S2 | | Do probe p9D11 | | P | 13q22 |
| D13S3 | | Do probe p9A7 | | P | 13q22-qter |
| D13S4 | | Do probe pIE8 | | P | 13q22-qter |
| D13S5 | | Do probe pHUB8 | | C | 13q12-q22 |
| D13S6 | | Do probe pHU10 | | C | 13q12-q22 |
| D13S7 | | Do probe pHU26 | | C | 13q12-q22 |
| D14S1 | | Do probe pAW101 | | C | 14q32 |
| D14S2 | | Do probe L1.48 | | P | 14 |

| | | | |
|---|---|---|---|
| D14S3 | Do probe G3 | P | 14 |
| D15S1 | Do probe pMS1-14 | P | 15 |
| D15Z1 | DNA segment, repetitive, probe pHSR-Sau3A | P | 15p |
| D16S1 | DNA segment, single copy, probe L1.36 | P | 16 |
| D16S2 | Do probe L1.19 | P | 16 |
| D16Z1 | DNA segment, repetitive, probe QP23 | P | 16 |
| D17S1 | DNA segment, single copy, probe pHF12.1 | P | 17pter-p13 |
| D18S1 | Do probe 12-62 | P | 18 |
| D18S3 | Do probe B74 | P | 18 |
| D18S4 | Do probe pMS2-12 | P | 18 |
| D19S1 | Do probe 5-14 | P | 19 |
| D19S2 | Do probe 4-8 | P | 19 |
| D19S3 | Do probe pA9 | P | 19 |
| D20S1 | Do probe 18BA | P | 20 |
| D20S2 | Do probe 46EA | P | 20 |
| D20S3 | Do probe 13.2 | P | 20 |
| D20S4 | Do probe pMS1-27 | P | 20 |
| D21S1 | Do probe pPW228C | P | 21 |

Contd. (*Appendix: Table VI: 1.2*)

| 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| D21S6 | | Do probe pPW237D | | P | 21 |
| D21S8 | | Do probe pPW245D | | P | 21 |
| D21S15 | | Do probe E8 | | P | 21 |
| D21S16 | | Do probe E9 | | P | 21 |
| D21S17 | | Do probe H8 | | P | 21 |
| D21S18 | | Do probe B1 | | P | 21 |
| D21S19 | | Do probe B3s | | P | 21 |
| D21S20 | | Do probe G5 | | P | 21 |
| D22S1 | | Do probe pMS3-18 | | P | 22pter-q13 |
| D22S2 | | Do probe P3-2 | | P | 22 |
| D22S3 | | Do probe 22-1 | | P | 22pter-q11.2 |
| D22S4 | | Do probe 22-2 | | P | 22q11.2-qter |
| D22S5 | | Do probe 22-3 | | P | 22q11.2-qter |
| D22S6 | | Do probe 22-4E | | P | 22q11.2-qter |
| D22S7 | | Do probe 22-5 | | P | 22q11.2-qter |
| D22S8 | | Do probe 22-6 | | P | 22q11.2-qter |
| DB | | double band salivary protein | | | LG4 |
| DC | | major histocompatibility complex, class II antigens | | C | 6p21.3 |
| DCE | | desmosterol-to-cholesterol enzyme | | P | 20 |
| DFN1 | | deafness, progressive | | P | X |

| | | | | | |
|---|---|---|---|---|---|
| DFN2 | | deafness, perceptive, congenital | | C | X |
| DFN3 | | deafness, conductive, with fixed stapes | | C | X |
| DGI1 | DGI | dentinogenesis imperfecta 1 | | C | 4 |
| DGS | | DiGeorge syndrome | | C | 22q11 |
| DHFR | | dihydrofolate reductase | 1.5.1.3 | P | 5 |
| DHTR | | dihydrotestosterone receptor | | C | Xp11-q11 |
| DIA1 | | diaphorase (NADH) (cytochrome b-5 reductase) | 1.6.2.2 | C | 22q13.31-qter |
| DIA4 | | diaphorase (NADH/NADPH) (cytochrome b-5 reductase) | 1.3.2.2 | C | 16q12-q21 |
| DIN | | diabetes insipidus, neurohypophyseal type | | C | X |
| DIR | | diabetes insipidus, renal | | C | X |
| DKC | | dyskeratosis congenita | | C | X |
| DM | | dystrophia myotonia | | C | 19 |
| DMD | MDD | muscular dystrophy, Duchenne type | | C | Xp21 |
| DNCM | | DNA associated with cytoplasmic membrane | | P | 9q12 |
| DNF1 | | DNA segment, numerous copies, probe pAX6 | | P | Xq26-qter |
| DNF2 | | Do probe pAX2 | | P | Xp21-p21 |

Contd. (*Appendix: Table VI: 1.2*)

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| DNF3, DNF4, DNF5, DNF6, DNF7, DNF8 | | DNA segment, numerous copies, probe pAX7, probe pAX1, probe pAX3, probe pAX19, probe pAX21, probe pAX22 | | P | Xq26-qter |
| DNL | | lysosomal DNAase | | P | 19pter-q13 |
| DTS | | diphtheria toxin sensitivity | | C | 5q15-qter |
| DXS1 | | DNA segment, single copy, probe 8EA | | P | Xcen-q1 |
| DXS2 | | Do probe 33HA | | P | Xp22-p21 |
| DXS3 | | Do probe p19-2 | | P | Xq11-q22 |
| DXS4 | | Do probe 12EA | | P | Xq22-q24 |
| DXS5 | | Do probe 14BA | | P | Xq22-q24 |
| DXS6 | | Do probe 32EA | | P | Xq24-qter |
| DXS7 | | Do probe L1.28 | | C | Xp11.3-p11 |
| DXS8 | | Do probe RB6 | | C | Xq24-q26 |
| DXS9 | | Do probe RC8 | | C | Xp22.3-q21 |
| DXS10 | | Do probe p6A | | P | Xq26-qter |
| DXS11 | | Do probe 22-33 | | P | Xq24-qter |
| DXS12 | | Do probe L2 | | P | Xq24-qter |
| DXS13 | | Do probe XUT3 | | P | Xq13-qter |
| DXS14 | | Do probe 58HA-1 | | P | Xp21-p11 |

| | | | |
|---|---|---|---|
| DXS15 | Do probe DX13 | P | Xq28-qter |
| DXS16 | Do probe pXUT23-2.1 | P | Xpter-p21 |
| DXS17 | Do probe S21/S9 | P | Xq21.3-q22 |
| DXS18 | Do probe pXQMGR1-22 | P | Xp22.3-p21.1 |
| DXS19 | Do probe pXQMGR1-22 | P | Xq25-qter |
| DXS20 | Do probe 735 | T | X |
| DXS22 | Do probe 3SA | P | Xp22.3-p22 |
| DXS23 | Do probe pX-4 | P | Xq26-qter |
| DXS24 | Do probe pX-5 | P | Xq26-qter |
| DXS25 | Do probe yHUB2 | P | Xq24-qter |
| DXS26 | Do probe pHU16 | P | Xq11-q22 |
| DXS27 | Do probe pX-18 | P | Xq26-qter |
| DXS28 | Do probe C7 | P | Xp22-p11 |
| DXS29 | Do probe DX4 | P | Xq28-qter |
| DXS30 | Do probe JP7 | P | Xq23-q26 |
| DXS31 | Do probe MIA | P | Xpter-p22.3 |
| DXS32 | Do probe M2C | P | Xp22-p11 |
| DXS33 | Do probe MN12 | P | Xq28-qter |
| DXS34 | Do probe RD6-1 | P | Xp11.3-p11 |
| DXS35 | Do probe RJ8 | P | Xq24-q28 |
| DXS36 | Do probe RA8 | P | Xq22-q24 |
| DXS37 | Do probe 30RIb | P | Xq25-qter |
| DXS38 | Do probe pXUT2-1.7 | P | Xpter-p13 |

Contd. (*Appendix: Table VI: 1.2*)

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| DXS39 | | Do probe pXUT22-3 | | P | Xpter-p21 |
| DXS40 | | Do probe pXUT1-5 | | P | Xq26-q28 |
| DXS41 | | Do probe p99-6 | | P | Xp21-cen |
| DXS42 | | Do probe p43-15 | | P | Xq24-qter |
| DXS43 | | Do probe pD2 | | P | Xp22.3-p21 |
| DXS44 | | Do probe Hx39 | | T | Xp22.1-p11 |
| DXS45 | | Do probe Hx120B | | T | Xq22-q26 |
| DXS46 | | Do probe Hx113 | | T | Xq22-q24 |
| DXS47 | | Do probe 83RId | | P | Xp22.3-p21 |
| DXS48 | | Do probe XBS81 | | P | Xq21-q25 |
| DXS49 | | Do probe 78RIc | | P | Xq25-qter |
| DXS50 | | Do probe 785 | | T | X |
| DXS51 | | Do probe 52A | | P | Xq27-qter |
| DXS52 | | Do probe St14 | | P | Xq26-q27 |
| DXS53 | | Do probe St16 | | P | Xq23-q26 |
| DXS54 | | Do probe St3 | | P | Xq13-q22 |
| DXS55 | | Do probe St10 | | P | Xq12-q13 |
| DXS56 | | Do probe 2.98 | | P | Xq12-q21.3 |
| DXS57 | | Do probe MGU90 | | P | Xq24-q26 |
| DXS58 | | Do probe MGU78 | | P | Xq24-q26 |
| DXS59 | | Do probe 2.9 | | P | Xq26-q27 |

| | | | |
|---|---|---|---|
| DXS60 | Do probe MGU72 | P | Xq11-q12 |
| DXS61 | Do probe St72 | P | Xq11-q2 |
| DXS62 | Do probe MGU22 | P | Xp11-q12 |
| DXS63 | Do probe pA29 | P | Xq24-qter |
| DXS64 | Do probe pG44 | P | Xq24-qter |
| DXS65 | Do probe pL3 | P | Xcen-q1 |
| DXS66 | Do probe p75-42 | P | Xq21-cen |
| DXS67 | Do probe pB24 | P | Xp22.3-p21 |
| DXS68 | Do probe pL1 | P | Xp22.3-p21 |
| DXS69 | Do probe p71-7A | P | Xpter-p22.3 |
| DXS70 | Do probe p18-55 | P | Xpter-p22.3 |
| DXS71 | Do probe 16BA | P | Xp21-cen |
| DXS72 | Do probe XBS59 | P | Xp11.4-q13 |
| DXS73 | Do probe XBS20 | P | Xq13-q21 |
| DXS74 | Do probe XBS13 | P | Xq21-q26 |
| DXS75 | Do probe XBS34 | P | Xq23-q25 |
| DXS76 | Do probe 77A | P | Xp22.3-p21 |
| DXS77 | Do probe XBS-59 | P | Xp11.4-q13 |
| DXYS1 | DNA segment, homologous on X and Y, probe pDP34 | P | Xq13-q22:Yp |
| DXYS2 | Do probe p7b | P | Xq12-q22;Y |
| DXYS3 | Do probe 31 | P | Xq12-q22;Y |
| DXYS4 | Do probe 1 | P | Xq12-q22;Y |

Contd. (*Appendix: Table VI: 1.2*)

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| DXYS5 | | Do probe 47C | | P | Xq12-q22;Y |
| DXYS6 | | Do probe 16 | | P | Xq12-q22;Y |
| DXYS7 | | Do probe 13d | | P | Xq12-q22;Y |
| DXYS8 | | Do probe 115 | | P | Xq12-q22;Y |
| DXYS9 | | Do probe 8j | | P | Xq12-q22;Y |
| DXYS10 | | Do probe 4la | | P | Xq22-q28;Y |
| DXYS11 | | Do probe 52d | | P | Xq22-q28;Y |
| DXYZ1 | | DNA segment, repetitive, homologous on X and Y, probe y35, x35A | | P | Xq12;Yq |
| DXZ1 | | DNA segment, repetitive, probe pXBR-1 | | C | Xp11-q13 |
| DXZ2 | | DNA segment, repetitive, probe pXQMGRI54 | | P | Xp11-q12 |
| DYS1 | | DNA segment, single copy, probe p49 | | P | Y |
| DYS2 | | Do probe p53 | | P | Y |
| DYS3 | | Do probe p52d | | P | Y |
| DYS4 | | Do probe p36a | | P | Y |
| DYS5 | | Do probe p27 | | P | Y |
| DYS6 | | Do probe p48 | | P | Y |
| DYS7 | | Do probe p50f1 | | P | Y |

| | | | | | |
|---|---|---|---|---|---|
| DYS8 | | Do probe p118 | | P | Y |
| DYS9 | | Do probe p40 | | P | Y |
| DYS10 | | Do probe pGV1 | | P | Y |
| DYZ1 | | DNA segment, repetitive, probe HS5 | | C | Yq11-q12 |
| DYZ2 | | Do probe pHY214 | | C | Yq12 |
| DYZ3 | | Do probe pAlphaRI-12 | | P | Y |
| DO | | Dombrock blood group | | T | 1 |
| E11S | | ECHO-11 virus sensitivity | | C | 19q |
| EBM | | epidermolysis bullosa, macular type | | P | X |
| EBR3 | | epidermolysis bullosa progressiva | | | LG8 |
| EBS1 | | epidermolysis bullosa simplex (Ogna) | | | LG2 |
| EDA | | ectodermal dysplasia, anhydrotic | | C | X |
| EFE | | endocardial fibroelastosis | | P | X |
| EGFR | S6 | epidermal growth factor receptor | | C | 7p13-p11 |
| EL1 | | elliptocytosis 1 (Rh linked) | | C | 1pter-p34 |
| ELA1 | | elastase 1 | 3.4.4.7 | P | 12 |
| EMD | | muscular dystrophy, Emery type | | C | X |
| ENO1 | | enolase 1 | 4.2.1.11 | C | 1pter-p36.13 |
| ENO2 | | enolase 1 | 4.2.1.11 | C | 12p11-qter |

Contd. (*Appendix: Table VI: 1.2*)

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| ERBA1 | | avian erythroblastic leukemia viral (v-*era*-a) oncogene homlog 1 | | C | 17p11-q21 |
| ERBB | | avian erythroblastic leukemia viral (v-*era*-b) oncogene homolog | | C | 7pter-q22 |
| ERCC | | excision repair complementing defective repair in Chinese hamster cells | | P | 1q |
| ERCM1 | | excision repair compensating in mouse cells | | P | 3 |
| ERCM2 | | excision repair complementing defective repair in mouse cells | | P | 13 |
| ESA4 | | esterase A4 | 3.1.1.1 | C | 11 |
| ESAT | | esterase activator | | P | 14 |
| ESB3 | | esterase B3 | 3.1.1.1 | P | 16 |
| ESD | | esterase D | 3.1.1.1 | C | 13q14.1 |
| F7 | | coagulation factor VII | | P | 13q34 |
| F9 | | coagulation factor IX (plasma thromboplastic component, Christmas disease) | | C | Xq26-q28 |
| F10 | | coagulation factor X | | P | 13q34 |
| F12 | HAF | coagulation factor XII (Hageman) | | P | 6pter-p23 |

| | | | | | |
|---|---|---|---|---|---|
| F13A | | coagulation factor XIII, A polypeptide | | P | 6 |
| FCP | | hemoglobin F cell production | | P | 11 |
| FDH | | formaldehyde dehydrogenase | 1.2.1.1 | P | 4p14-qter |
| FES | | feline sarcoma viral (v-fes) oncogene homolog | | C | 15q25-q26 |
| FGA and FGB | | fibrinogen, A alpha and B beta polypeptide | | C | 4q21-q31 |
| FGDY | | faciogenital dysplasia | | C | X |
| FGG | | fibrinogen, gamma polypeptide | | C | 4q21-q31 |
| FH | | fumarate hydratase | 4.2.1.2 | C | 1q42.1 |
| FHC | HC | hypercholesterolemia, familial | | C | 19 |
| FMS | | McDonough feline sarcoma viral (v-*fms*) oncogene homolog | | P | 5 |
| FN1 | | fibronectin identified with monoclonal antibodies | | C | 2 |
| FN2 | | fibronectin identified with non-monoclonal antibodies | | P | 11 |
| FN3 | | fibronectin-cell surface | | C | 8 |
| FPGS | | folylpolyglutamate synthetase | | P | 9cen-q34 |
| FRA2Q116 | | fragile site, folic acid type, fra (2) (q11.2) | | C | 2q11.2 |
| FRA2Q136 | | Do fra (2) (q13) | | C | 2q13 |

Contd. (*Appendix: Table VI: 1.2*)

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| FRA3P146 | | Do fra (3) (p14l) | | P | 3p14 |
| FRA6P236 | | Do fra (6) (p23) | | P | 6p23 |
| FRA6Q266 | | Do fra (6) (q26) | | C | 6q26 |
| FRA7P116 | | Do fra (7) (p11.2) | | C | 7p11.2 |
| FRA8Q226 | | Do fra (8) (q22.3) | | C | 8q22.3 |
| FRA9P216 | | Do fra (9) (p21.1) | | P | 9p21.1 |
| FRA9Q326 | | Do fra (9) (q32) | | P | 9q32 |
| FRA10Q236 | | Do fra (10) (q23.3) | | C | 10q23.3 |
| FRA10Q256 | | fragile site, BUdR type, fra (10) (q25.2) | | C | 10q25.2 |
| FRA11Q136 | | fragile site, folic acid type, fra (11) (q13.3) | | C | 11q13.3 |
| FRA11Q236 | | Do fra (11) (q23.3) | | P | 11q23.3 |
| FRA12Q136 | | Do fra (12) (q13.1) | | C | 12q13.1 |
| FRA16P126 | | Do fra (16) (p12.3) | | C | 16p12.3 |
| FRA16Q226 | | fragile site, distamycin A tvpe, fra (16) (q22.1) | | C | 16q22.1 |
| FRA16Q236 | | fragile site, type unknown, fra (16) (q23) | | C | 16q23 |
| FRA17P126 | | fragile site, distamycin A type, fra (17) (p12) | | C | 17p12 |

| | | | | | |
|---|---|---|---|---|---|
| FRA20P116 | | fragile site, folic acid type, fra (20) (p11.23) | | C | 20p11.23 |
| FRAXQ266 | | fragile site, type unknown, fra (X) (q26) | | C | Xq26 |
| FRAXQ276 | | fragile site, folic acid type, fra (X) (q27.3), (macroorchidism, mental retardation) | | C | Xq27.3 |
| FTH | | ferritin heavy polypeptide | | P | 19 |
| FTL | | ferritin light polypeptide | | P | 19 |
| FUCA1 | FUCA | alpha-L-fucosidase 1, tissue | 3.2.1.51 | C | 1p34 |
| FUCA2 | | alpha-L-fucosidase 2, plasma | 3.2.1.52 | P | 4 |
| FUSE | | polykaryocytosis promoter | | P | 10 |
| Fy | | Duffy blood group | | C | 1q12-q21 |
| G6PD | | glucose-6-phosphate dehydrogenase | 1.1.1.49 | C | Xq28 |
| GAA | | alpha-glucosidase, acid | 3.2.1.20 | C | 17q22-q25 |
| GALE | | UDP-galactose-4-epimerase | 5.1.3.2 | C | 1pter-p32 |
| GALK | | galactokinase | 2.7.1.6 | C | 17q21-q22 |
| GALT | | galactose-1-phosphate uridylyltransferase | 2.7.7.12 | C | 9p21-p13 |
| GANAB | | alpha-glucosidase, neutral AB | 3.2.1.20 | P | 11q13-qter |
| GANC | | alpha-glucosidase, neutral C | 3.2.1.20 | P | 15 |
| GAPD | | glyceraldehyde-3-phosphate dehydrogenase | 1.2.1.12 | C | 12p13 |

Contd. (*Appendix: Table VI: 1.2*)

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| GBA | | beta-glucosidase, acid | 3.2.1.45 | C | 1q |
| GC | | group-specific component | | C | 4q11-q13 |
| GCFI | | growth control factor 1 | | P | 7 |
| GCG | | glucagon | | P | 2 |
| GCTG | | gamma-glutamylcyclotransferase | 2.3.2.4 | P | 7pter-p14 |
| GDH | | glucose dehydrogenase | 1.1.1.47 | C | 1pter-p36.13 |
| GH1 and GH2 | GH | growth hormone 1 and 2 | | C | 17q22-q24 |
| GL | | parotid salivary glycoprotein | | | LG4 |
| GLA | | alpha-galactosidase | 3.2.1.22 | C | Xq21-q24 |
| GLAT | | galactose enzyme activator | | P | 2 |
| GLB1 | | beta-galactosidase 1 | 3.1.1.23 | C | 3p21-cen |
| GLB2 | | beta-galactosidase 2 | 3.2.1.23 | P | 22 |
| GLO1 | | glyoxalase I | 4.4.1.5 | C | 6p21.3-21.2 |
| GLYB | | glycine B complementing | | P | 8q21.1-qter |
| GOT1 | | glutamic-oxaloacetic transaminase 1, soluble | 2.6.1.1. | C | 10q25.3-26.1 |
| GOT2 | | Do 2, mitochondrial | 2.6.1.1 | C | 16p12-q22 |
| GPD1 | | glycerol-3-phosphate dehydrogenase | 1.1.1.8 | P | 12 |
| GPI | | glucose phosphate isomerase | 5.3.1.9 | C | 19p13.2-q13 |
| GPT | | glutamic-pyruvate transaminase | 2.6.1.2 | | LG2; 8q13-qter or16pter-p11 |

| | | | | | |
|---|---|---|---|---|---|
| GPX1 | | glutathione peroxidase 1 | 1.11.1.9 | C | 3p13-q12 |
| GRL | | glucocorticoid receptor | | P | 5 |
| GRP78 | | glucose regulated protein (MW 78K) | | P | 9q |
| GSAS | | glutamate gamma-semialdehyde synthetase | | P | 10 |
| GSR | | glutathione reductase | 1.6.4.2 | C | 8p211 |
| GST | GST1 | glutathione S-transferase, isozyme 1 or 3 | 2.5.1.18 | C | 11 |
| GUK1, GUK2 | | guanylate kinase 1 and 2 | 2.7.4.8 | C | 1q32-q42, 1 |
| GUSB | | beta-glucuronidase | 3.2.1.31 | C | 7cen-q22 |
| GUSM | | mouse beta-glucuronidase modifier | | P | 19 |
| H | | H blood group antigen | | C | 19 |
| H1 | | H1 histone | | C | 7q22;q32-q36* |
| H2A and H2B | | H2A and H2B histone | | C | 7q22;q32-q36* |
| H3 and H4 | | H3 and H4 histone | | C | 7q22;q32-q36* |
| HADH | | hydroxyacyl-CoA dehydrogenase | 1.1.1.35 | P | 7 |
| HAGH | | hydroxyacyl glutathione hydrolase | 3.1.2.6 | P | 16 |
| HBA1 and HBA2 | | haemoglobin, alpha 1 and 2 | | C | 16pter-p12 |
| HBB | | haemoglobin, beta | | C | 11p15 |
| HBBP1 | | haemoglobin, beta-like pseudogene 1 | | C | 11p15 |

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*Inconsistent assignment.

Contd. (*Appendix: Table VI: 1.2*)

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| HBBP2 | | haemoglobin, beta-like pseudogene 2 | | C | 11p15 |
| HBD | | haemoglobin, delta | | C | 11p15 |
| HBE1 and HBE2 | | haemoglobin, epsilon 1 and 2 | | C | 11p15 |
| HBG1 and HBG2 | | haemoglobin, gamma A and G | | C | 11p15 |
| HBZ | | haemoglobin, zeta | | C | 16pter-p12 |
| HBZP1 | | haemoglobin, zeta-like pseudogene | | C | 16pter-p12 |
| HCVS | | human coronavirus sensitivity | | P | 15q11-qter |
| HEMA | | haemophilia A, procoagulant component of Factor VIII | | C | Xq26-qter |
| HEMB | | haemophilia B | | C | X |
| HEXA | | hexosaminidase A (alpha subunit) | 3.2.1.30 | C | 15q22-q25.1 |
| HEXB | | hexosaminidase B (beta subunit) | 3.2.1.30 | C | 5q13 |
| HHG | | hypergonadotropic hypogonadism | | | LG10 |
| HK1 | | hexokinase 1 | 2.7.1.1 | C | 10 |
| HLA-A, B and C | | major histocompatibility complex, class I antigens | | C | 6p21.3 |
| HLA-D and DR | | major histocompatibility complex, class II antigens | | C | 6p21.3 |
| HOAC | | hypoacusis-2 (recessive) | | | LG8 |
| HP | | haptoglobin | | C | 16q21-q22 |

| | | | | |
|---|---|---|---|---|
| HPDR | hypophosphatemia, vitamin D resistant rickets | | C | X |
| HPRT | hypoxanthine phosphoribosyltransferase | 2.4.2.8 | C | Xq26-q27 |
| HPT | hypoparathyroidism | | P | X |
| HRAS1 | Harvey rat sarcoma 1 viral (v-Ha-*ras*1) oncogene homolog | | C | 11p15.5-p15.1 |
| HRAS2 | Do, 2 viral (v-Ha-*ras*2) oncogene homolog | | P | X |
| HSAS | hydrocephalus, stenosis of the aqueduct of Sylvius | | C | X |
| HSGA | glycoprotein alpha2 HS | | | LG1 |
| HSH | hypomagnesemia, secondary hypocalcemia | | C | X |
| HTOR | 5-hydro-tryptamine oxygenase regulator | | P | 21 |
| HV1S | herpes simplex virus type 1 susceptibility | | I | 3 or 11 |
| HYA | Y histocompatibility antigen | | P | Y |
| HYB | Y histocompatibility antigen regulator | | P | X |
| HYC | Y histocompatibility antigen receptor | | P | X |

Contd. (*Appendix: Table VI: 1.2*)

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| IDH1 | | isocitrate dehydrogenase 1, soluble | 1.1.1.42 | C | 2q32-qter |
| IDH2 | | isocitrate dehydrogenase 2, mitochondrial | 1.1.1.42 | C | 15q21-qter |
| IDUA | | alpha-L-iduronidase | 3.2.1.76 | P | 22pter-q11 |
| IF1 | | interferon 1, fibroblast | | P | 2p23-qter |
| IF2 | | interferon 2, fibroblast | | P | 5p |
| IFA | IFL | interferon, alpha (leukocyte) | | C | 9pter-p13 |
| IFB | IFF | interferon, beta (fibroblast) | | C | 9p24-p13 |
| IFG | | interferon, gamma | | C | 12q24.1 |
| IFR | | interferon production regulator | | P | 16 |
| IFRC | | interferon receptor | | C | 21q21-qter |
| IGHA1 and 2 | | immunoglobulin alpha 1 and 2 polypeptide | | C | 14q32.3 |
| IGHD and E | | immunoglobulin delta and epsilon polypeptide | | C | 14q32.3 |
| IGHEP2 | | immunoglobulin epsilon polypeptide pseudogene 2 | | C | 9 |
| IGHG1 and 2 | | immunoglobulin gamma 1 and 2 polypeptide | | C | 14q32.3 |

| | | | |
|---|---|---|---|
| IGHG3 and 4 | immunoglobulin gamma 3 and 4 polypeptide | C | 14q32.3 |
| IGHGPI | immunoglobulin gamma polypeptide pseudogene 1 | P | 14q32.3 |
| IGHM | immunoglobulin mu polypeptide | C | 14q32.3 |
| IGHV | immunoglobulin heavy polypeptide, variable region | C | 14q32.3 |
| IGK | immunoglobulin kappa polypeptide, region not specified | C | 2 |
| IGKC | Do constant region | C | 2 |
| IGKV | Do variable region | C | 2p12 |
| IGLC | immunoglobulin lambda polypeptide, constant region | C | 22q11 |
| IGLV | Do variable region | T | 22q11 |
| IHG | iris hypoplasia with glaucoma | P | X |
| Ii | Ii blood group | | LG7 |
| IL2 | interleukin 2 | C | 4 |
| IMD1 | immunodeficiency 1, combined, progressive (Bruton agammaglobulinemia) | P | X |
| IMD2 | immunodeficiency 2 (Wiskott-Aldrich syndrome) | C | X |

*Contd.* (*Appendix: Table VI: 1.2*)

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| IMD3 | | immunodeficiency 3, with increased IgM | | P | X |
| IMD4 | | immunodeficiency 4, thymic epithelial hypoplasia | | C | X |
| INS | | insulin | | C | 11p15 |
| INT1 | | mammary tumor virus integration site (v-*int* 1) oncogene homolog | | P | 12pter-q14 |
| IP | | incontinentia pigmenti | | P | X |
| IS | | immune suppression | | P | 6p21.3 |
| ITPA | | inosine triphosphatase (nucleoside triphosphate pyrophosphatase) | 3.6.1.19 | C | 20p |
| Jk | | Kidd blood group | | C | 2 |
| K | | Kell blood group | | | LG3 |
| KAL | | Kallmann syndrome | | P | X |
| KAR | | alpha-keto acid (aromatic) reductase | | P | 12p |
| KRAS1 | | Kirsten rat sarcoma 1 viral (v-Ki-*ras* 1) oncogene homolog, processed pseudogene | | P | 6p23-q12 |
| KRAS2 | | Kirsten rat sarcoma 2 viral | | C | 12 |

| | | | | |
|---|---|---|---|---|
| | (v-Ki-*ras* 2) oncogene homolog | | | |
| LARS | leucyl-tRNA synthetase | 6.1.1.4 | C | 5pter-q11 |
| LCAT | lecithin-cholesterol acyltransferase | 2.3.1.43 | C | 16q21-q22 |
| LCH | lentil agglutinin binding | | P | 14 |
| LDHA | lactate dehydrogenase A | 1.1.1.27 | C | 11p12.03-p12.08 |
| LDHB | lactate dehydrogenase B | 1.1.1.27 | C | 12p12.2-p12.1 |
| Le | Lewis blood group | | C | 19 |
| LEU5 | leukocyte antigen 5 | | P | 4 |
| LEU7 | leukocyte antigen 7 | | P | 11 |
| LGS | Langer-Giedion syndrome | | C | 8q |
| LHB | luteinising hormone beta-subunit | | C | 19 |
| LIPA | lipase A | | C | 10 |
| LIPB | lipase B | | P | 16 |
| LOX | lysyl oxidase; cutis laxa-X; Ehlers-Danlos V | | C | X |
| Lu | Lutheran blood group | | C | 19 |
| M130 | external membrane protein (MW 130K) | | P | 10 |
| M195 | external membrane protein (MW 195K) | | P | 14 |
| M7VI | baboon M7 virus receptor | | P | 19 |

Contd. (*Appendix: Table VI: 1.2*)

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| MAA | | microphthalmia or anophthalmia, associated anomalies | | C | X |
| MANA | | alpha-mannosidase A | 3.2.1.24 | C | 15q11-qter |
| MANB | | alpha-mannosidase B, lysosomal | 3.2.1.24 | C | 19pter-q13 |
| MAOA | | monoamine oxidase | 1.4.3.4 | P | X |
| MARS | | methionine tRNA synthetase | 6.1.1.10 | P | 12 |
| MDH1 | | malate dehydrogenase NAD (soluble) | 1.1.1.37 | C | 2p23 |
| MDH2 | | Do NAD (mitochondrial) | 1.1.1.37 | C | 7p13-q22 |
| MDU1 | | antigen identified by monoclonal antibody 4F2 | | P | 11q |
| MDU2 and 3 | | antigen identified by monoclonal antibody A1G3 and A3D8 | | P | 11p15-p11 |
| ME1 | | malic enzyme, soluble | 1.1.1.40 | C | 6q12 |
| MEI1 | | Do antibody RIB-19 | | P | 11 |
| MEI2 | | Do antibody S4.7.13 | | P | 11 |
| MEI3 | | Do antibody B37.4 | | P | 11 |
| MEI4 | | Do antibody 1G10 | | P | 11 |
| MEI5 | | Do antibody IH4 | | P | 11 |

| | | | |
|---|---|---|---|
| MEI6 | Do antibody 3B9 | P | 11 |
| MEI7 | Do antibody 6G5 | P | 11 |
| MEI8 | Do antibody 4C10 | P | 11 |
| MEI9 | Do antibody VIM-2 | P | 11 |
| MEI10 | Do antibody VIM-7 | P | 11 |
| MEI11 | Do antibody VIM-8 | P | 11 |
| MEI12 | Do antibody MI/N1 | P | 11 |
| MEI13 | Do antibody UJ-308 | P | 11 |
| MEI14 | Do antibody VIM-D5 | P | 11 |
| MEI15 | Do antibody FMC-10 | P | 11 |
| MEI16 | Do antibody B4.3 | P | 11 |
| MEN2 | multiple endocrine neoplasia, type II | P | 20p12.2 |
| MER1 | antigen identified by monoclonal antibodies 4C11, 5B1 | P | 11pter-p13 |
| MER2 | Do antibodies 1D12, 2F7 | P | 11p15 |
| MER3 | Do antibody 3C5 | P | 11p12-p11 |
| MER4 | Do antibody 6C12 | P | 11p12-p11 |
| MF4 | metacarpal 4-5 fusion | C | X |
| MFI1 | antigen (MW 75K) identified by monoclonal antibody Mab4 | P | 11 |
| MFI2 | antigen p97 (melanoma associated) identified by monoclonal | P | 3 |

Contd. (*Appendix: Table VI: 1.2*)

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
|  |  | antibodies 133.2 and 96.5 |  |  |  |
| MFI3 |  | antigen (glycoprotein, MW 86K) identified by monoclonal antibody 2B2 |  | P | 21 |
| MFI4 |  | antigen (glycoprotein, MW 145K) identified by monoclonal antibody 2B2 |  | P | 21 |
| MIC1 |  | antigen identified by monoclonal antibody W6/34 |  | P | 11pter-p13 |
| MIC2X | MIC2 | Do antibody 12E7 |  | P | Xpter-p22.3 |
| MIC2Y | MIC2 | Do antibody 12E7 |  | P | Ypter-q11 |
| MIC3 |  | Do antibody 602-29 |  | P | 12 |
| MIC4 |  | Do antibody F10.44.2 |  | P | 11pter-p13 |
| MIC5 |  | Do antibody R1 |  | P | Xq13-qter |
| MIC6 |  | Do antibody H207 |  | P | 17q21-qter |
| MIC7 |  | Do antibody 28.3.7 |  | P | 15q11-q22 |
| MIC8 |  | Do antibody TRA1.10 |  | P | 11q |
| MIC9 |  | Do antibody 4D12 |  | P | 11q |
| MIC11 |  | Do antibody 163A5 |  | P | 11pter-p13 |

| | | | | |
|---|---|---|---|---|
| MIC12 | Do antibody 30.2A8 | | P | 15q11-qter |
| MN | MN blood group | | C | 4q28-q31 |
| MNK | Menkes syndrome | | C | Xp11-q11 |
| MOS | Moloney murine sarcoma viral (v-*mos*) oncogene homolog | | C | 8q22 |
| MPI | mannose phosphate isomerase | 5.3.1.8 | C | 15q22-qter |
| MRBC | monkey red blood cell receptor | | P | 6 |
| MSD | microcephaly with spastic diplegia (Paine syndrome) | | P | X |
| MSK1 | antigen identified by monoclonal antibody AJ9 | | P | 1p22-cen |
| MSK2 | Do antibody T87 | | P | 1q32-qter |
| MSK3 | Do antibody M68 | | P | 12p |
| MSK4 | Do antibody A123 | | P | 12q |
| MSK5X | Do antibody 013 | | P | Xpter-p22 |
| MSK5Y | Do antibody 013 | | P | Y |
| MSK6X | Do antibody F21 | | P | Xpter-p22 |
| MSK6Y | Do antibody F21 | | P | Y |
| MSS | Marinesco-Sjogren syndrome | | | LG10 |
| MTR | tetrahydropteroylglutamate methyltransferase | 2.1.1.13 | P | 1 |
| MYB | avian myeloblastosis viral (v-*myb*) oncogene homolog | | C | 6q15-q24 |

Contd. (*Appendix: Table VI: 1.2*)

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| MYC | | avian myelocytomatosis viral (v-*myc*) oncogene homolog | | C | 8q24 |
| MYH1 | | myosin heavy polypeptide, skeletal muscle, adult 1 | | P | 17pter-p11 |
| MYH2 | | Do muscle, adult 2 | | P | 17pter-p11 |
| MYH3 | | Do muscle, embryonic | | P | 17pter-p11 |
| MYH4 | | Do muscle, adult | | P | 7cen-q11.2 |
| NAGA | | alpha-N-acetylgalactosaminidase | 3.2.1.49 | P | 22q13-qter |
| NARS | | asparaginyl-tRNA synthetase | 6.1.1.22 | P | 18 |
| NDP | | Norrie disease (pseudoglioma) | | C | X |
| NF1 | | neurofibromatosis 1 (von Recklinghausen disease) | | C | 19 |
| NGF | | nerve growth factor | | P | 1pter-p21 |
| NHCP1 | | non-histone chromosome protein 1 | | P | 16 |
| NHCP2 | | non-histone chromosome protein 2 | | P | 7 |
| NKRS | | natural killer cell radiation sensitivity | | P | X |
| NM | | neutrophil migration | | P | 7q22-qter |
| NP | | nucleoside phosphorylase | 2.4.2.1 | C | 14q131 |
| NPS1 | | nail patella syndrome 1 | | C | 9q3 |

| | | | | |
|---|---|---|---|---|
| NRAS | neuroblastoma RAS viral (v-*ras*) oncogene homolog | | C | 1p31.1-p22.1 |
| NYS | nystagmus | | C | X |
| OA | ocular albinism | | C | Xp22 |
| OCRL | oculocerebrorenal syndrome of Lowe | | C | X |
| OIAS | 2′,5′-oligoisoadenylate synthetase | | P | 11 |
| OPD | otopalatal digital syndrome | | P | X |
| OPEM | ophthalmoplegia, external, with myopia | | P | X |
| ORM | orosomucoid | | C | 9q |
| OTC | ornithine carbamoyltransferase | 2.1.3.3 | C | X |
| P | P blood group | | T | 6 |
| PA | parotid acidic salivary protein | | | LG4 |
| PAH | phenylalanine hydroxylase | 1.14.16.1 | P | 12 |
| PAIS | phosphoribosylaminoimidazole synthetase | 6.3.3.1 | P | 21 |
| PB | parotid basic protein | | | LG4 |
| PEPA | peptidase A | 3.4.11.* or<br>3.4.13.* | C | 18q23 |
| PEPB | peptidase B | 3.4.11.* or<br>3.4.13.* | C | 12q21 |
| PEPC | peptidase C | 3.4.11.* or<br>3.4.13.* | C | 1q |

Contd. (Appendix: Table VI: 1.2)

| 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| PEPD | | peptidase D | 3.4.13.9 | C | 19pter-q13 |
| PEPS | | peptidase S | 3.4.11.* or 3.4.13.* | C | 4p12-q12 |
| PFGS | | phosphoribosylformyglycinamidine synthetase (formylglycinamide ribotide) | 6.3.5.3 | P | 14 |
| PFKL | | phosphofructokinase, liver-type | 2.7.1.11 | C | 21q22 |
| PFKM | | phosphofructokinase, muscle type | 2.7.1.11 | P | 1cen-q32 |
| PFKP | PFKF | phosphofructokinase, platelet type | 2.7.1.11 | C | 10pter-p11.1 |
| PGAMA | | phosphoglycerate mutase A | 2.7.5.3 | P | 10q25.3-q26.1 |
| PGD | | phosphogluconate dehydrogenase | 1.1.1.44 | C | 1pter-p36.13 |
| PGFT | | phosphoribosylglycinamide formyltransferase | 2.1.2.2 | P | 14q22-qter |
| PGK | | phosphoglycerate kinase | 2.7.2.3 | C | Xq13 |
| PGM1 | | phosphoglucomutase 1 | 2.7.5.1 | C | 1p221 |
| PGM2 | | phosphoglucomutase 2 | 2.7.5.1 | C | 4p14-q12 |
| PGM3 | | phosphoglucomutase 3 | 2.7.5.1 | C | 6q12 |
| PGP | | phosphoglycollate phosphatase | 3.1.3.18 | C | 16p13-p12 |
| PHP | | panhypopituitarism | | P | X |
| PI | | alpha-1-antitrypsin (protease inhibitor) | | C | 14q24.3-q32.2 |

| | | | | | |
|---|---|---|---|---|---|
| PIF | | parotid isolectric focusing protein | | | LG4 |
| PKM2 | | pyruvate kinase | 2.7.1.40 | C | 15q22-qter |
| PLA | | plasminogen activator | | P | 6 |
| PLG | | plasminogen | | P | 4 |
| PM | | parotid middle band protein | | | LG4 |
| PMD | | Pelizaeus-Merzbacher disease | | C | X |
| POMC | POC | propiomelanocortin (adrenocorticotropin/ beta-lipotropin) | | C | 2p23 |
| PP | | pyrophosphatase (inorganic) | 3.6.1.1 | C | 10q11.1-q24 |
| PPAT | | phosphoribosyl pyrophosphate amidotransferase | 2.4.2.14 | P | 4pter-q21 |
| PPB | | post parotid basic protein | | | LG4 |
| PR | | proline rich salivary protein | | | LG4 |
| PRGS | | phosphoribosylglycinamide synthetase | 6.3.4.13 | C | 21q22 |
| PRL | | prolactin | | P | 6p23-q12 |
| PRPS | | phosphoribosyl pyrophosphate synthetase | 2.7.6.1 | C | Xq22-q26 |
| PS | | parotid (protein) size variant | | | LG4 |
| PSF1 | | protein spot in 2-D gels (MW 24K) | | P | Xq12-q28 |
| PSF2, 3 and 4 | | protein spot in 2-D gels (MW 27K) | | P | Xpter-q13 |

Contd. (*Appendix: Table VI: 1.2*)

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| | | (MW 37K), (MW 40K) | | | |
| PSP | | phosphoserine phosphatase | 3.1.3.3 | C | 7pter-q22 |
| PTC | | phenylthiocarbamide tasting | | | LG3 |
| PTH | | parathyroid hormone | | C | 11pter-p11 |
| PUT1 | | polypeptide m44p in lymphocyte membrane | | P | 6 |
| PVS | | poliovirus sensitivity | | C | 19 |
| PIF | | parotid isoelectric focusing protein | | | LG4 |
| PWS | PWS1 | Prader-Willi syndrome | | C | 15q11 |
| PYHG1 | | protein spot in 2-D gels (MW 218K) | | P | 10 |
| PYHG2 | | protein spot in 2-D gels (MW 178K) | | P | 11 |
| PYHG3 | | protein spot in 2-D gels (MW 106K) | | P | 7 |
| PYHG4 | | protein spot in 2-D gels (MW 104K) | | P | X |
| PYHG5 | | protein spot in 2-D gels (MW 90K) | | P | 6pter-21 |
| PYHG6 | | protein spot in 2-D gels (MW 85K) | | P | 12 |
| PYHG7 | | protein spot in 2-D gels (MW 82K) | | P | 1p32-qter |
| PYHG8 | | protein spot in 2-D gels (MW 80K) | | P | 7 |
| PYHG9 | | protein spot in 2-D gels (MW 79K) | | P | 1p32-qter |
| PYHG10 | | protein spot in 2-D gels (MW 77K) | | P | 12 |
| PYHG11 | | protein spot in 2-D gels (MW 74K) | | P | 15 |

| | | | | |
|---|---|---|---|---|
| PYHG12 | protein spot in 2-D gels (MW 55K) | | P | X |
| PYHG13, 14 and 15 | protein spot in 2-D gels (MW 40K, 37K, 35K) | | P | 9 |
| PYHG16 | protein spot in 2-D gels (MW 38K) | | P | 9 |
| PYHG17 | protein spot in 2-D gels (MW 32K) | | P | X |
| PYHG18 | protein spot in 2-D gels (MW 31K) | | P | 10 |
| PYHG19 | protein spot in 2-D gels (MW 12K) | | P | 12 |
| PYHG20 | protein spot in 2-D gels (MW 10K) | | P | 15 |
| PYHG21 | protein spot in 2-D gels (MW 10K) | | P | 1p32-qter |
| PYHG22 | protein spot in 2-D gels (MW <10K) | | P | 1p32-qter |
| PYK | phosphorylase kinase, liver, glycogen storage disease VIII | 2.7.1.38 | C | X |
| QDPR | quinoid dehydrogenase reductase | 1.6.5.1 | P | 4 |
| RACH | acetylcholinesterase derepressor | | P | 2 |
| RAF1 and 2 | murine leukemia viral (v-*raf*-2) oncogene homolog 1 and 3 | | P | 3 and 4 |
| RB1 | retinoblastoma 1 | | C | 13q14.1 |
| Rd | Radin blood group | | P | 1p34-p22.1 |
| RDRC | RD114 virus receptor | | P | 19 |
| REN | renin | | P | 1p21-qter |
| Rh | Rhesus blood group | | C | 1p36-p34 |
| RN5S | 5S RNA | | C | 1q42-q43 |

Contd. (*Appendix: Table VI: 1.2*)

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| RNR | | ribosomal RNA | | C | 13p12, 14p13, 15p12, 21p12, 22p12 |
| RNU1 | | U1 small nuclear RNA | | C | 1p36 |
| RP2 | | retinitis pigmentosa 2 | | C | X |
| RPE | | ribulose-5-phosphate-3-epimerase | 5.1.3.1 | C | 2q32-qter |
| RPS14 | EMTB | ribosomal protein S14 | | P | 5q31-q35 |
| RS | | retinoschisis | | C | Xp |
| S1 | | lethal antigen 1 | | C | 11pter-p13 |
| S2 | | lethal antigen 2 | | P | 11q13-qter |
| S3 | | lethal antigen 3 | | P | 11pter-p13 |
| S4 | | species antigen | | P | 11 |
| S5 | | surface antigen 5 (MW 45K) | | P | 6 |
| S7 | | surface antigen (chromosome 7) 2 | | P | 7 |
| S8 | | surface antigen (chromosome 12) 1 | | P | 12 |
| S9 | | surface antigen (chromosome 17) 1 | | P | 17 |
| S10 | | surface antigen (X linked) 1 | | P | Xq26-q28 |
| S12 | | surface antigen (X linked) 3 | | P | X |
| S13 | | surface antigen (chromosome 22) | | P | 22 |
| S14 | | surface antigen (chromosome 21) | | P | 21 |
| SB | | secondary B-cell response | | C | 6p21.3 |

| | | | | | |
|---|---|---|---|---|---|
| SBMA | | spinal and bulbar muscular atrophy | | C | X |
| Sc | | Scianna blood group | | C | 1p34-p32 |
| SCA1 | | spinal cerebellar ataxia (olivopontocerebellar ataxia) | | C | 6 |
| SDH | | succinate dehydrogenase 1 (1 of 2 polypeptides) | 1.3.99.1 | P | 1p22.1-qter |
| Se | | ABH secretion | | C | 19 |
| SEDL | | spondyloepiphyseal dysplasia, late | | P | X |
| Sf | | Stoltzfus blood group | | P | 4 |
| SHMT | | serine hydroxymethyl transferase | 2.1.2.1 | C | 12q12-q14 |
| SIDS | | sulfoiduronate sulfatase | | C | X |
| SIS | | simian sarcoma viral (v-*sis*) oncogene homolog; structural gene for platelet derived growth factor | | C | 22q12.3-13.1 |
| SK | D1S3 | chicken viral (v-SK) oncogene homolog | | P | 1q12-qter |
| SOD1 | | superoxide dismutase 1, soluble | 1.15.1.1 | C | 21q22.1 |
| SOD2 | | superoxide dismutase 2, mitochondrial | 1.15.1.1 | C | 6q21 |
| SORD | | sorbitol dehydrogenase | 1.1.1.14 | P | 15pter-q21 |
| SPH1 | | spherocytosis | | P | 8 |
| SRBC | | sheep red blood cell receptor | | P | 6 |

*Contd.* (*Appendix: Table VI: 1.2*)

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| SRC | | avian sarcoma viral (v-*src*) oncogene homolog | | P | 20 |
| SRD1 | | specific reading disability | | P | 19 |
| Ss | | Ss blood group | | C | 4q28-q31 |
| SST | | somatostatin | | P | 3q28 |
| STS | | steroid sulfatase (microsomal) | | C | Xpter-p22.3 |
| TBG | | thyroxin binding globulin | | C | X |
| TCD | | choroideremia (progressive tapeto-choroidal dystrophy) | | C | X |
| TDD | | testicular 17, 20-desmolase deficiency | | C | X |
| TDF | | testis determining factor(s) | | P | Ypter-p11.2 |
| TF | | transferrin | | | LG1 |
| TFRC | | transferrin receptor | | C | 3q23-qter |
| TG | | thyroglobulin | | P | 8 |
| TK1 | | thymidine kinase (soluble) | 2.7.1.21 | C | 17q21-q22 |
| TK2 | | Do (mitochondrial) | 2.7.1.21 | P | 16 |
| TKC | | torticollis, keloids, cryptorchidism, and renal dysplasia | | P | Xq28-qter |
| TPI1 | | triosephosphate isomerase 1 | 5.3.1.1 | C | 12p13 |

| | | | | |
|---|---|---|---|---|
| TPI2 | triosephosphate isomerase 2 | 5.3.1.1 | T | 12 |
| TRM1 and 2 | methionine tRNA initiator 1 and 2 | | P | 6p23-q12 |
| TRY1 | trypsin 1 | 3.4.4.4 | P | 7q22-qter |
| TYS | sclerotylosis | | P | 4 |
| UGP1 | UDP glucose pyrophosphorylase 1 | 2.7.7.9 | C | 1q21-q22 |
| UGP2 | UDP glucose pyrophosphorylase 2 | 2.7.7.9 | P | 2 |
| UMPK | uridine monophosphate kinase | 2.7.4.* | C | 1p32 |
| UP | uridine phosphorylase | 2.4.2.3 | C | 7 |
| UPS | uroporphyrinogen I synthase | 4.3.1.8 | C | 11q23-qter |
| URIC | uridine biosynthetic mutant C (OPRT/ODC activity) | | P | 3cen-q21 |
| VDI | vesicular stomatitis virus defective interfering particle suppressor | | P | 16 |
| VMD1 | macular dystrophy, vitelliform, atypical | | | LG2 |
| WAGR | Wilms' tumor—aniridia, genitourinary abnormalities, and mental retardation triad | | C | 11p13 |
| WARS | tryptophanyl-tRNA synthetase | 6.1.1.2 | C | 14q21-qter |
| WS1 | Waardenberg syndrome, type 1 | | T | 9q34 |
| Xg | Xg blood group | | C | Xpter-p22.3 |
| Xk | Kell blood group precursor | | C | Xpter-p21 |
| XM | Xm(a) antigen | | P | X |

*Contd. (Appendix· Table VI· 1.2)*

| *1* | *2* | *3* | *4* | *5* | *6* |
|---|---|---|---|---|---|
| XPAC | | fast kinetic complementation DNA repair in xeroderma pigmentosum, group A | | P | 1q |

---

[1] This catalog has been compiled from this and past Human Gene Mapping workshops. Only marker assignments and linkages have been included that are supported by factual genetic evidence. For a discussion of individual genetic markers refer to reports of the chromosomes to which the markers have been assigned.

[2] Symbols for genetic markers follow guidelines of the Nomenclature Committee presented at the Edinburgh workshop (Shows *et al.*, 1979) and amended at the Oslo and this workshop. It is recommended that these guidelines be followed for maintaining uniformity among gene symbols.

[3] Where changes in gene symbols have oocurred, the previous symbols are indicated.

[4] An assignment by two independent laboratories is considered confirmed and listed as (C). A provisional assignment by one laboratory is listed as (P) ; an assignment based on suggestive evidence is listed as tentative (T), and an assignment inconsistent between two laboratories is listed as (I).

[5] Chromosomal assignment and the smallest most consistent region are indicated. Genes that are linked but which have not been assigned to a specific chromosome are listed and follow the linkage group designations of the committee on unassigned linkage groups (this conference, 1984).

[6] Symbols not yet approved by the International Standing Committee for Human Chromosome Nomenclature.

### Plate 1: Somatic metaphase plates in plants.

Top Left: *Codiaeum variegatum* (high number of chromosomes of similar size). Top Right: *Euphorbia heterophylla* (2n=22, all short chromosomes of equal size). Middle Left: Variation in *Havorthia* sp. with nine long and three short chromosomes. Middle Right: *Aloe davyana* (eight long and six short). Bottom Left: *Apium graveolens* variation plate with 2n=21 chromosomes. Inset: *Amaryllis* sp. (2n=22). Bottom Right: *Funkia ovata* (2n=60, 10L+50S).

### Plate 2: Divisional stages.

Top Left: *Zephyranthes mesochloa* (2n=24) polysomaty, showing two somatic cells with different chromosome numbers. Top Right: *Ornithogalum thyrsifolium* (2n=24), same. Middle Left: Meiotic configurations—multivalent formation. Middle Right: Meiotic configurations—regular bivalent formation. Bottom Left: Meiotic irregularities including lagging, inversion bridge with fragments. Bottom Right: Irregular separation of bivalents.

### Plate 3: Effects of X-rays on somatic chromosomes.

Top Left: Stickiness in metaphase. Top Right: Laggards in anaphase. Middle Left and Inset: Multipolar formation in anaphase. Middle Right: Irregular separation in metaphase. Bottom Left: Sticky bridge and spiralisation in anaphase. Bottom Right: Multinucleate condition.

### Plate 4: Effects of chemicals on somatic chromosomes.

Top Left: Diplochromatid. Top Right: C-mitosis with normal diploid and polyploid cells. Middle Left: Irregular grouping in metaphase. Middle Right: Irregular separation of chromosomes. Bottom Left: Localised fragment. Bottom Right: Multipolar formation.

### Plate 5: Divisional stages (*Continued*).

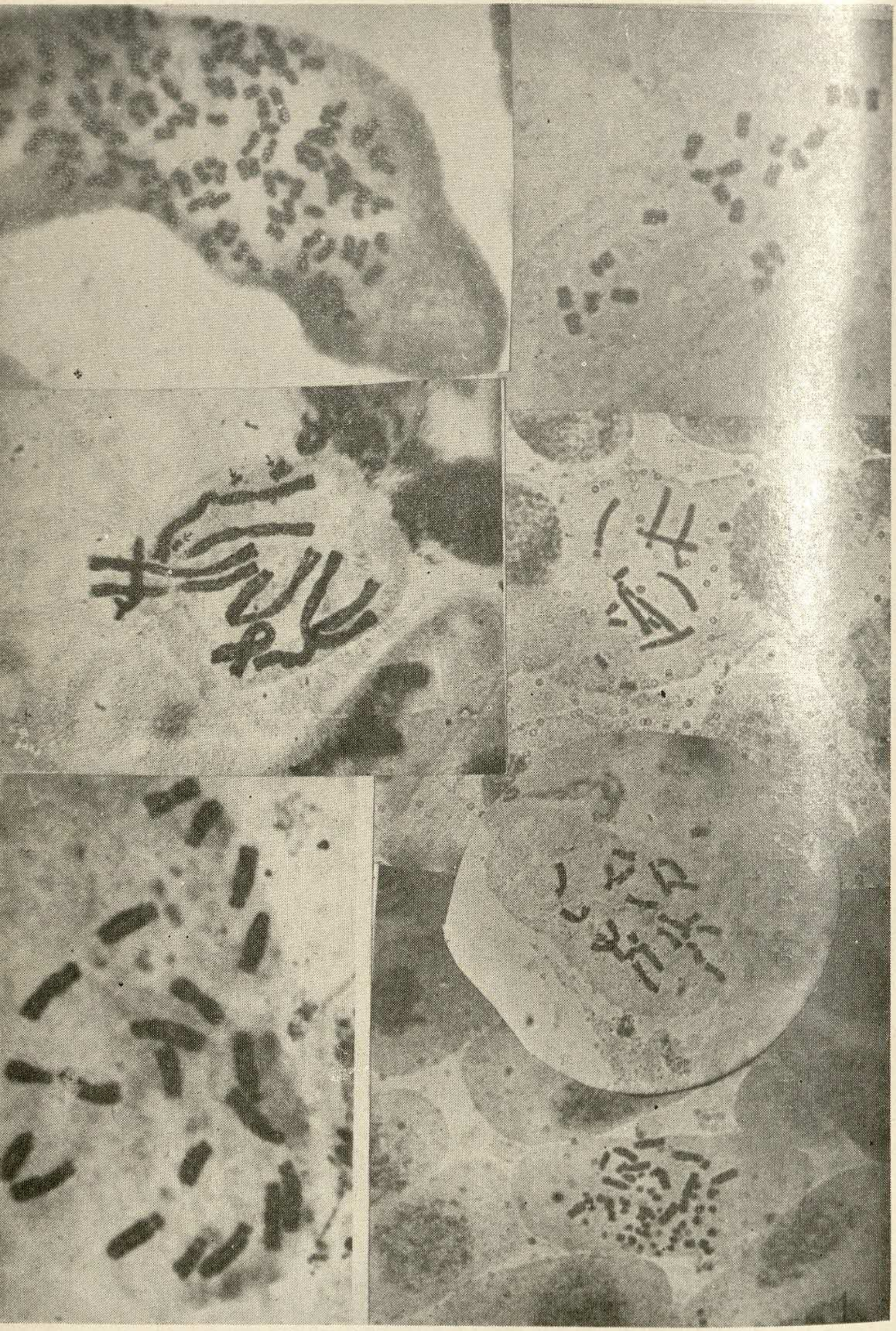
Top Left: Somatic chromosomes of swine (2n=40×Y male) from peripheral blood culture (Courtesy: S. Makino). Top Right: Somatic chromosomes of snake through peripheral blood culture. Middle: Anaphase showing accessory chromosomes in *Allium stracheyii* (2n=16+1–8B). Inset: Somatic metaphase showing accessory chromosomes in *Smilax macrophylla*. Bottom: *Chorthippus parallelus*, diplotene of male meiosis, eight autosomal bivalents and one×univalent with lampbrush like appearance (Courtesy: B. John).

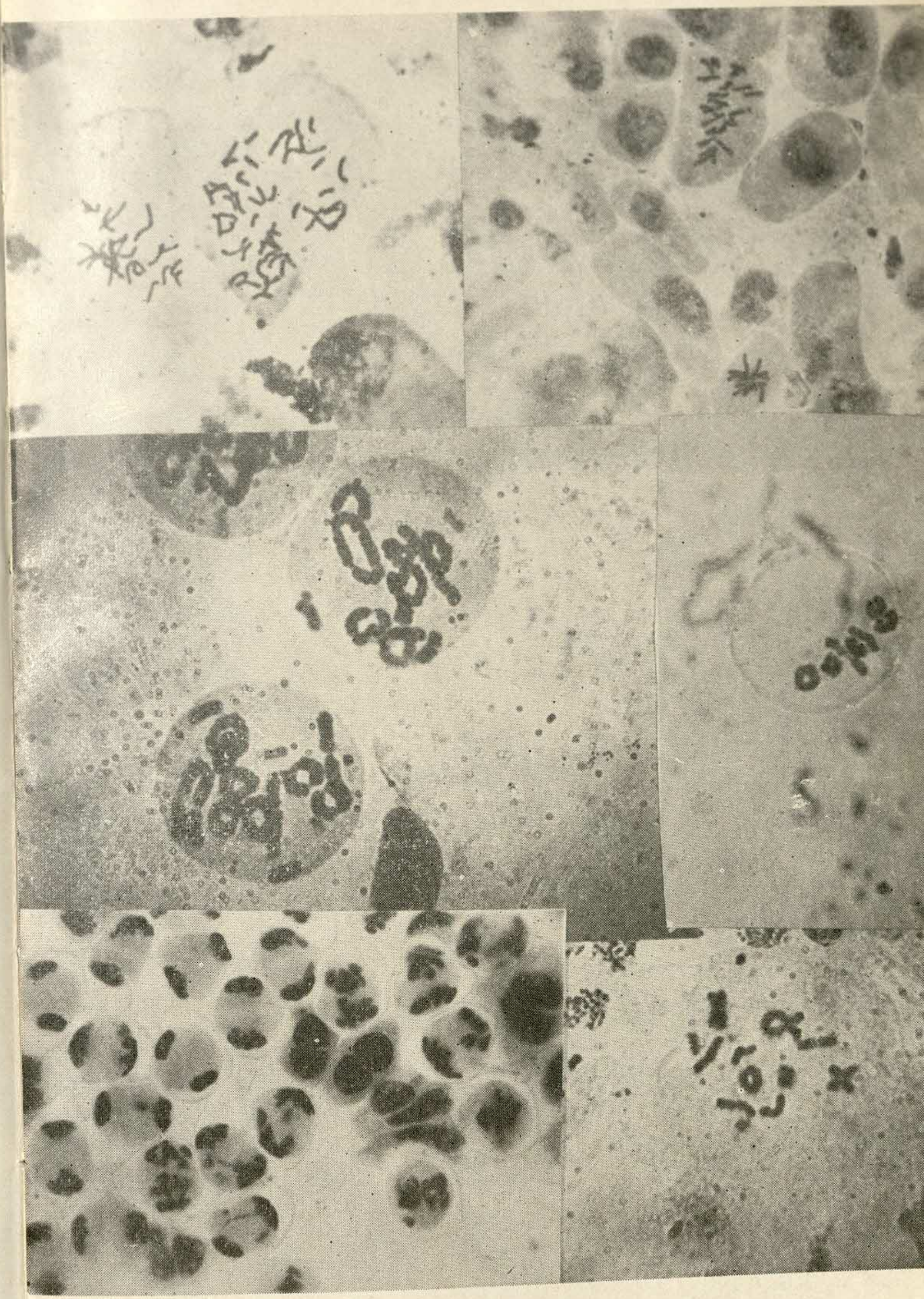
### Plate 6: Human chromosomes.

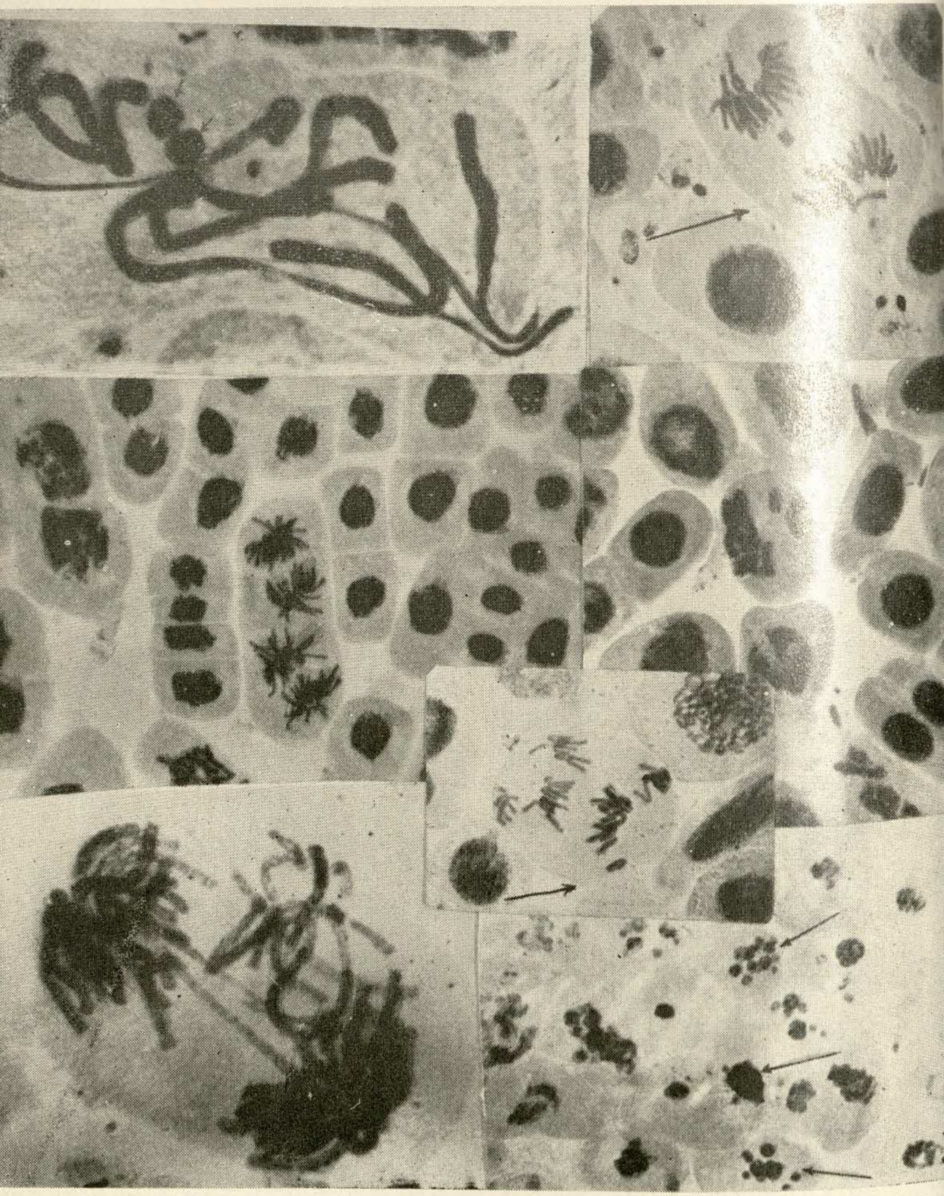
Top: A normal human somatic metaphase, after Giemsa staining showing 2n=46 chromosomes. Bottom: A cancerous cell in a human male, showing 46 chromosomes, but with structural alterations, after G-banding.

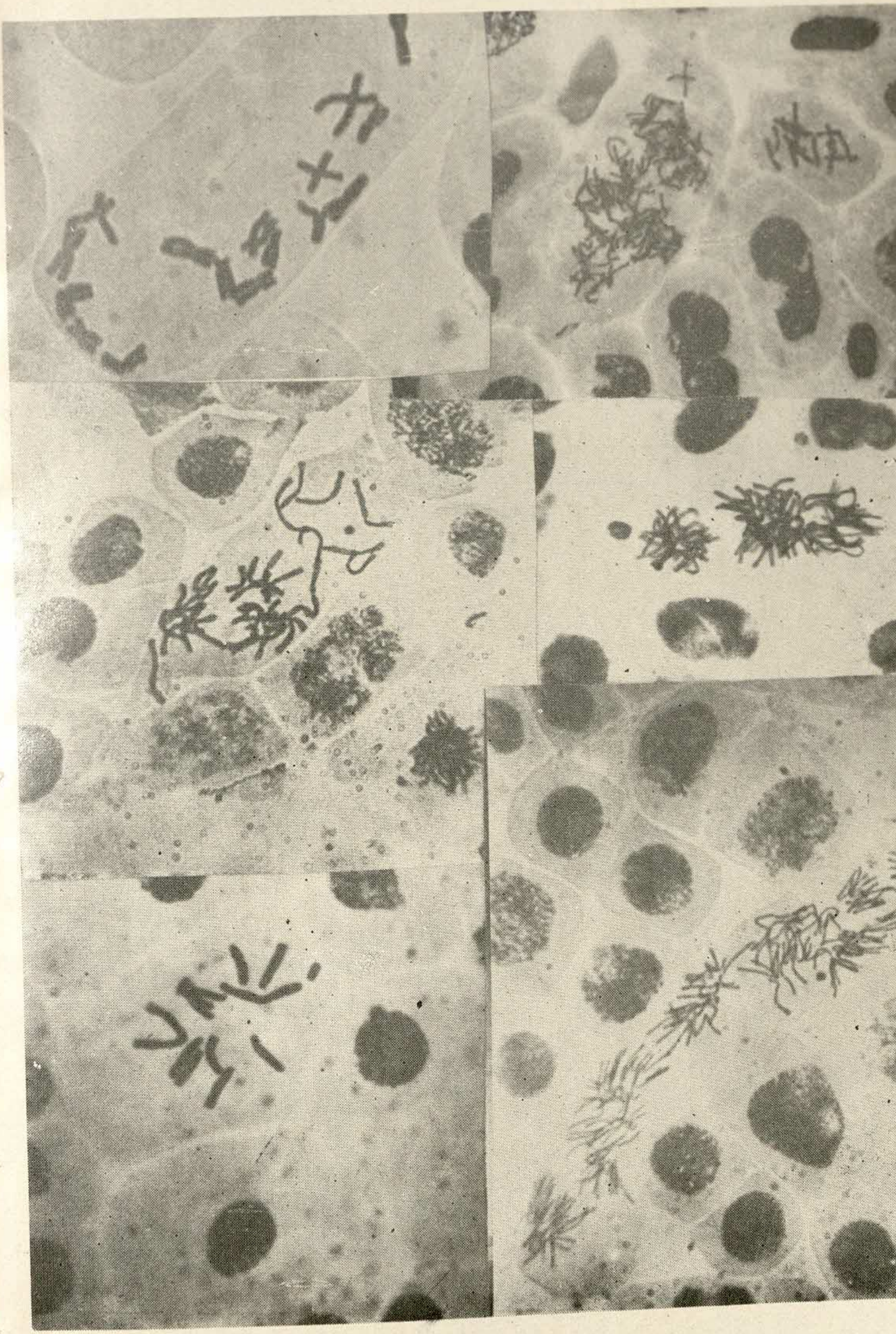
### Plate 7: Karyotypes of human chromosomes after Q-banding, arranged according to size and morphology.

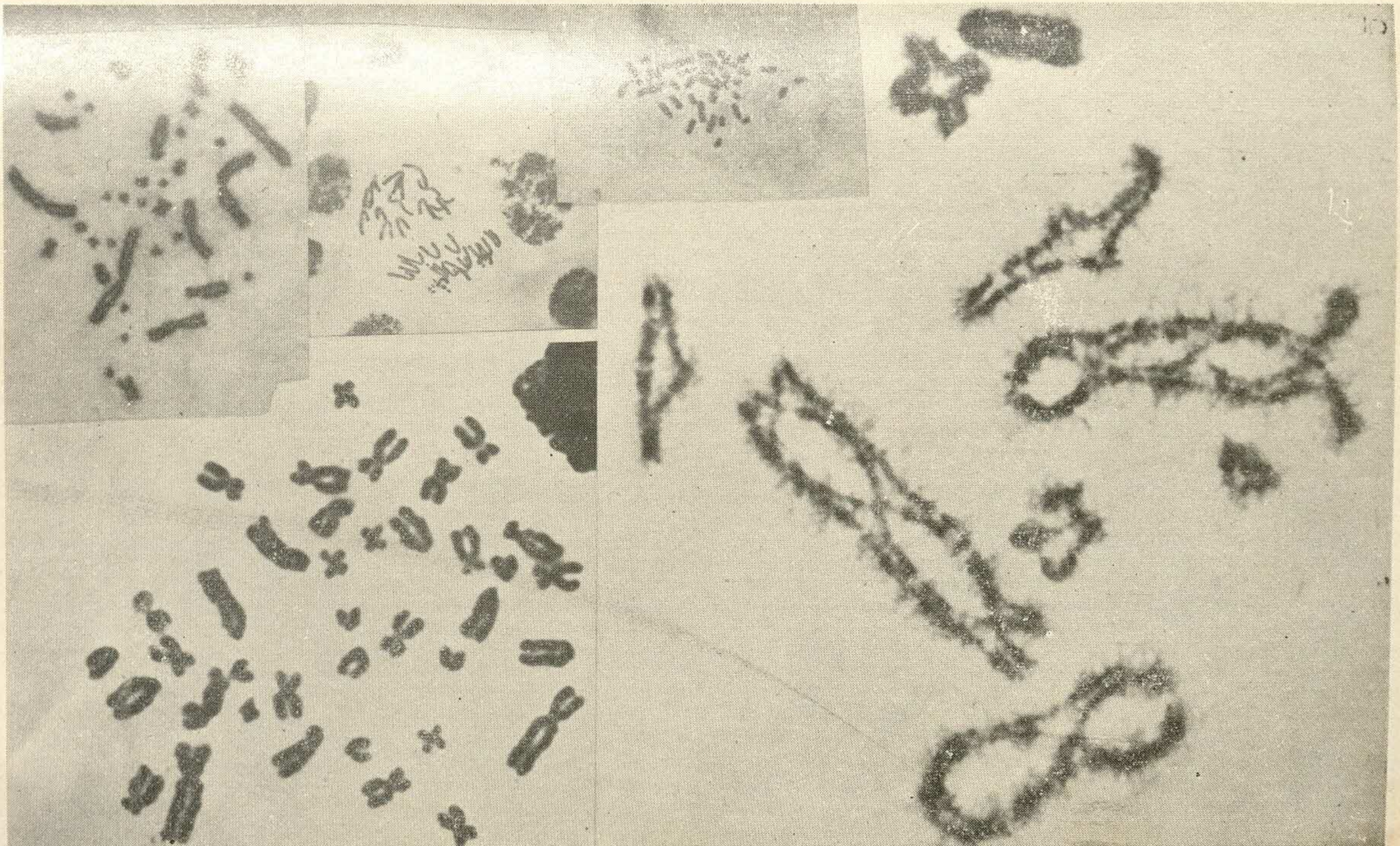
Top: A normal male with 46 chromosomes. Bottom: A somatic cell showing 47 chromosomes with trisomy for chromosome No. 4.







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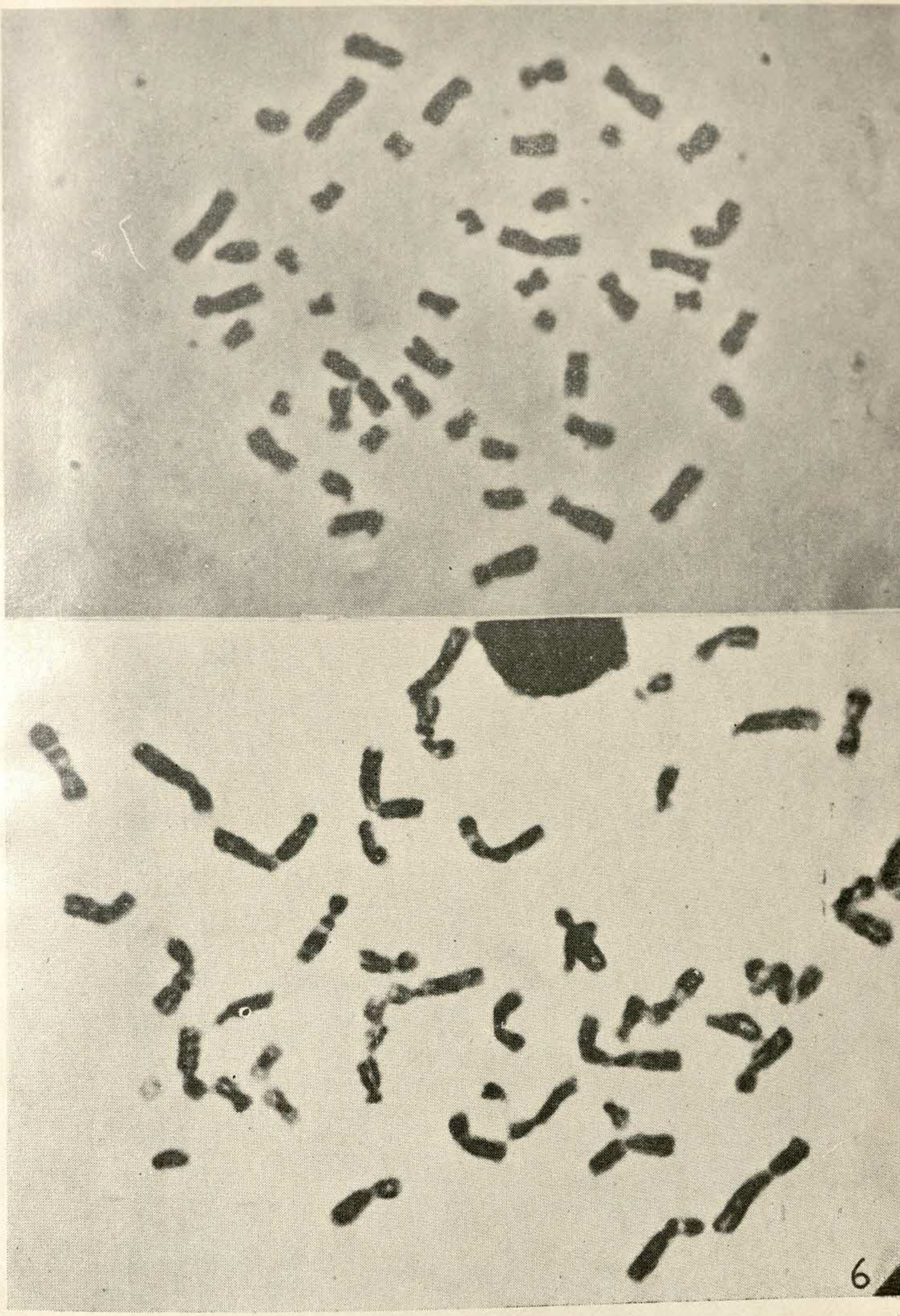
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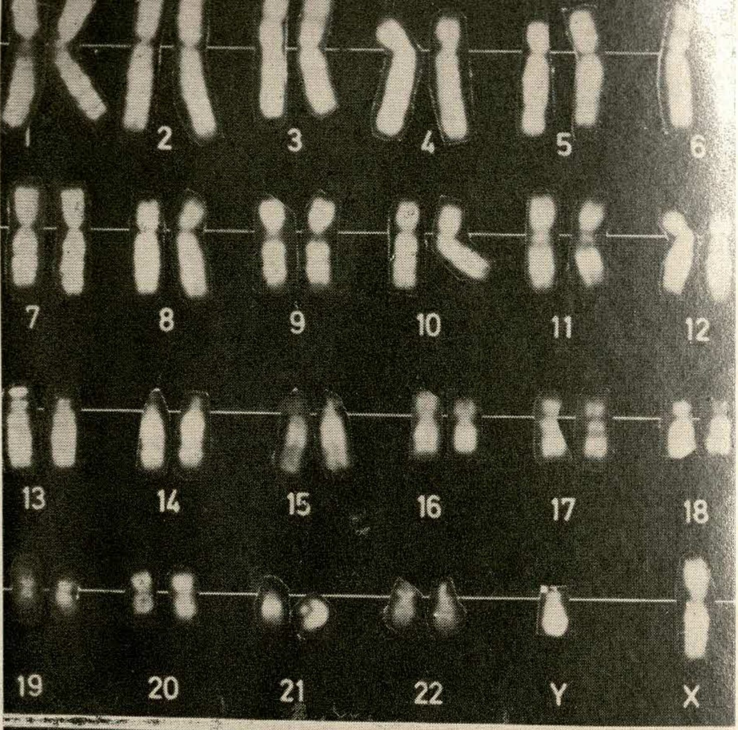
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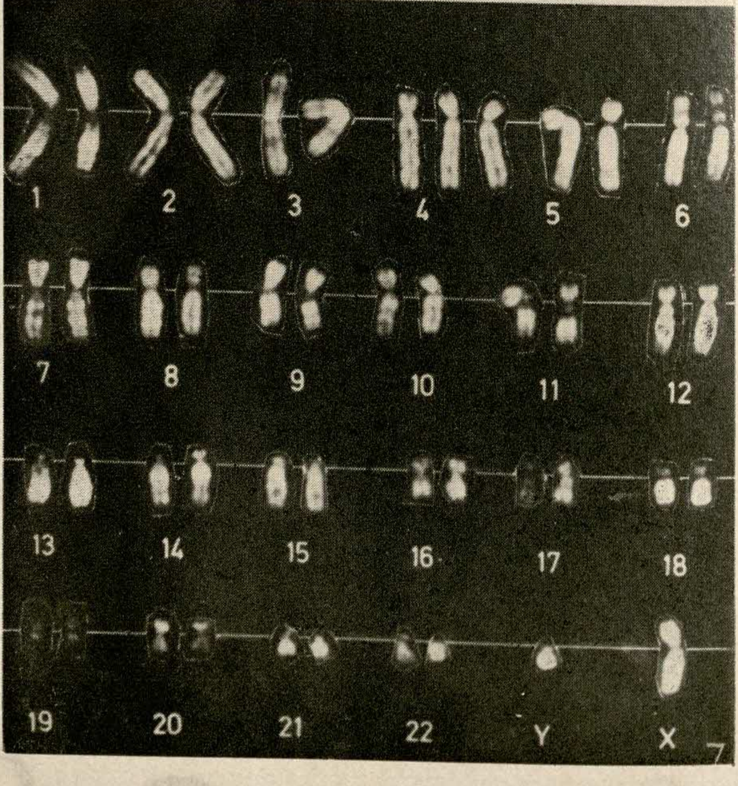
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